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Vol. XXIV, No. 11, November, 1941

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The Journal of Dairy Science is issued monthly. Subscription is by the volume and one volume is issued per year.

Manuscripts should be typewritten and carefully revised before submission to T. S. Sutton, The Ohio State University, Columbus, Ohio. Twenty-five reprints will be furnished gratis to authors. Cost of additional reprints and reprint order blank will be submitted with proof.

The use of material published in the Journal is encouraged and a liberal policy will be followed concerning reproduction of articles with proper notation as to source.

Subscriptions. Price; $6.00 per volume in United States and Canada; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Correspondence regarding business policies of the Journal should be addressed to the Secretary-treasurer, R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Entered as second-class matter April 13, 1934 at the postoffice at Lancaster, Pa., under the act of March 3, 1879.

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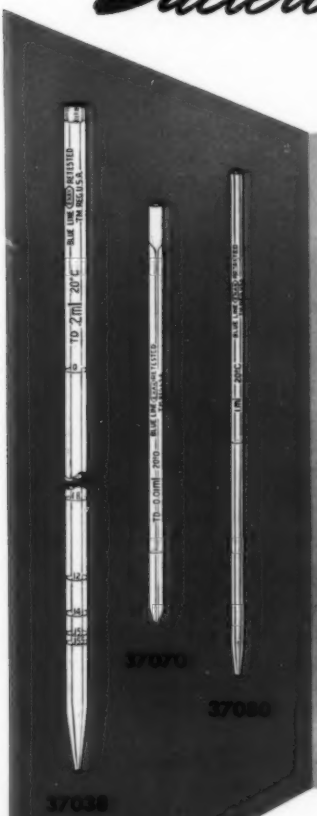

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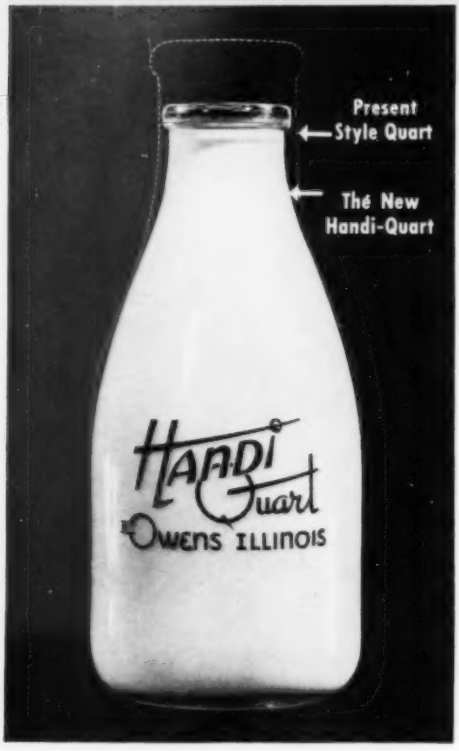

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JOURNAL OF DAIRY SCIENCE

VOLUME XXIV NOVEMBER, 1941 NUMBER 11

PRESERVATION OF BOVINE SPERMATOZOA IN YOLK–CITRATE DILUENT AND FIELD RESULTS FROM ITS USE

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INTRODUCTION

Scherstén (11) found appreciable quantities of citrates in the sexual gland secretions of man and animals and stated that a considerable portion of the buffer capacity of the semen was due to citrates. Others, including Slowtzoff (12), Huggins and Johnson (5), and Goldblatt (3), working with human beings, and McKenzie and co-workers (6), working with the boar, have analyzed semen, and their work shows that it contains considerable quantities of phosphates and carbonates. Willett (14) recently has determined the buffer coefficient curves for representative samples of the semen of the bull, the stallion, man, and the dog. The peaks of buffer capacity of bull semen coincide with the pH levels at which citrates, phosphates, and carbonates would be effective as buffers.

Scherstén (11) found that addition of sodium citrate to a Ringer-phosphate solution at the rate of 30 to 60 mg. per 100 cc. increased the longevity of spermatozoa suspended therein. Earlier Gray (4) had used sodium citrate to disperse spermatozoa agglutinated by metallic ions. However, Fleig (2) and Dubincik (1) have reported that citrate anion had a deleterious effect on sperm.

In light of these several investigations it was thought worth-while to develop a buffer mixture for the preservation of bull spermatozoa which would correspond closely to the buffers of semen to be used with egg yolk in place of the phosphate suggested by Phillips (8) and Phillips and Lardy (9), and which had given such splendid results in investigations by us (15).

LABORATORY STUDIES

An M/15 solution of sodium citrate and an M/15 solution of potassium di-hydrogen phosphate were added to egg yolk in such amounts as to make the resulting mixture contain about equal parts of egg yolk and buffer with

Received for publication June 12, 1941.

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the pH adjusted at 6.75. When first used it was noted that the new citrate buffer dispersed the fat globules and other materials in the yolk so that the resulting mixture was clear in appearance. When using the microscope to examine a sample of semen diluted with this mixture one could readily discern the individual sperm; on the other hand, when using the yolk-phosphate diluent it was necessary to further dilute the mixture with a clear diluter before the individual sperm could be distinguished.

Since this property was lost when the proportion of citrate was reduced by a small amount it was decided to use only enough potassium di-hydrogen phosphate as was compatible with satisfactory storage. Investigation showed that the phosphate buffer was not necessary for satisfactory results in the storage of semen when egg yolk was also used. Consequently, in the investigations herein reported the yolk-citrate diluent was composed of equal parts of fresh egg yolk and an M/15 solution of sodium citrate. Four or five parts of this diluent were added to one part of semen for the storage studies.

Comparisons were then undertaken to determine the relative value of the yolk-citrate diluent and the yolk-phosphate diluent in preserving the motility of spermatozoa under standard storage conditions. All the semen used in this investigation was collected from the dairy bulls in the Cornell University herd with the new-type artificial vagina (10). The semen was divided into equal portions immediately after collection. One-half was diluted at the rate of 1 part of semen to 4 or 5 parts of the yolk-citrate diluent. The other half was diluted at the same rate with yolk-phosphate dilutent made after the directions of Phillips and Lardy (9).

The diluted samples were divided into 0.5 cc. portions and placed in small culture tubes. These tubes were then wrapped individually with paper and put into larger test tubes. The larger tubes were then placed in controlled-temperature water baths for cooling. Cooling was accomplished by changing the tubes from one bath to another at definite intervals. In these studies the semen was cooled from room temperature to the storage temperature of 5° C. at the rate of 5° C. per 10 minutes. After storage the samples were warmed at the rate of 10° C. per 10 minutes. Willett (14) has shown that, while one must carefully control the rate of cooling of semen for best results, the rate of warming the sample is apparently of little importance.

Each sample of semen was examined for motility at 37° C. in a microscope stage incubator immediately after collection and after 2, 4, 6, 8, and 10 days of storage. With the yolk-phosphate diluent it was necessary to further dilute the samples with SGC–2 diluter (Milovanov (7)) so that the spermatozoa might be readily seen. Studies of the buffer coefficient curves of this diluter showed that it possessed no appreciable buffer capacity and did not influence the pH of the stored semen plus yolk-diluent when added

to it. Estimations of motility were made on the basis of the proportion of actively motile spermatozoa and were expressed as per cent to the nearest unit of 10. Willett (14) has shown that this method of estimation of motility gives highly repeatable results.

RESULTS OF LABORATORY STUDIES

In table 1 is presented a summary of the studies comparing the motility of the spermatozoa during storage in the citrate and phosphate diluents. By the analysis of variance technique (13), no significant differences were

TABLE 1

Comparison of motility of spermatozoa during storage in yolk-phosphate and yolk-citrate diluents at 5° C. and without mineral oil

Time stored	Sample pairs	Motility means			Probabilities for differences between means
		Before storage	After storage		
			Phosphate	Citrate	
days	*No.*	%	%	%	
2	19	71	47	50	$> .05$
4	19	72	38	40	$> .05$
6	17	72	26	35	$< .01$
8	19	72	23	32	$< .01$
10	16	72	13	22	$< .01$

detected after 2 and 4 days of storage. After longer intervals of storage the differences were highly significant in favor of the citrate diluent. In addition, it was noted that, especially after 6 and 8 days storage, the spermatozoa were more active and many more showed progressive motility in the citrate than in the phosphate diluent.

In table 2 is presented a summary of the pH values of the semen-yolk-buffer mixtures before and after storage. It can be readily seen that there was practically no difference between the average pH values of the two mixtures either before or after storage for different intervals.

TABLE 2

Comparison of pH of semen during storage in yolk-phosphate and yolk-citrate diluents at 5° C. and without mineral oil

Time stored	Sample pairs	pH means			
		Before storage		After storage	
		Phosphate	Citrate	Phosphate	Citrate
days	*No.*	*pH*	*pH*	*pH*	*pH*
2	18	6.74	6.71	6.62	6.64
4	19	6.74	6.71	6.55	6.54
6	17	6.73	6.70	6.54	6.50
8	19	6.73	6.70	6.50	6.45
10	15	6.72	6.70	6.45	6.44

Although the average pH values of the mixtures before storage were about the same, the values for the semen in the citrate buffer were more variable than those for the phosphate buffers, for their coefficients of variation were 1.12 per cent and 0.73 per cent, respectively. This can be explained by the lower buffer capacity of the citrate diluent at these pH levels, with the result that the pH of the semen, which is quite variable, would have a greater influence on the pH of the citrate than on the phosphate diluent.

FIELD STUDIES AND RESULTS

Since the citrate buffer appeared to excel the phosphate buffer when used with egg yolk for the preservation of semen during extended storage, an experiment was designed to compare the fertility of spermatozoa stored for various intervals in the two diluents. This experiment was undertaken in cooperation with the Seneca (Seneca County, New York) Cooperative Cattle Breeders' Association, Inc., and all of the inseminations were carried out by H. K. Fuller.

The semen was collected and handled in a manner already described (15). Alternate ejaculates from each bull were preserved in the citrate and the phosphate diluents and then used for breeding after various intervals of storage. Semen from five bulls was used and the results for each bull are reported separately in table 3. Bulls number I and II are Holsteins, the others are Guernseys.

Save for the first twenty-four-hour period, the use of the citrate and phosphate diluents gave comparable results until the fifth day when the citrate gave slightly better results. However, this latter difference was not significant, nor were any of the other differences between the results obtained with the yolk-citrate and yolk-phosphate diluents significant when analysed with chi-square in a two by two table. Furthermore, when the values for all of the time intervals were totalled, the numbers of services required per conception with the semen preserved in the two diluents were practically identical. As far as these fertility studies go they tend to bear out the results of the storage studies which showed no significant differences until the sixth day and after. If the fertility studies had been extended over a longer period of storage the superiority of the citrate diluent which was indicated from the results of inseminations during the fifth day might have been established as real.

SUMMARY

1. An M/15 solution of sodium citrate mixed in equal amounts with fresh egg yolk produced a diluent which dispersed the fat globules and other material in the yolk so that when semen was diluted with it the individual spermatozoa could be readily seen upon microscopic examination.

2. The yolk-citrate diluent and the yolk-phosphate diluent were ap-

TABLE 3

Breeding results in the Seneca Cooperative Cattle Breeders' Association when comparing the yolk-phosphate and yolk-citrate diluents

Bull	Age of semen used (hours)														Services per conception
	0–24		24–48		48–72		72–96		96–120		120–144		Totals		
	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	
Yolk-phosphate diluent															
I	10	6	8	6	14	7	6	4	11	7	2	1	51	31	1.65
II	1	1	9	6	4	3	6	4	6	3	9	4	35	21	1.67
III	5	5	9	5	10	8	8	7	4	2	....	....	36	27	1.33
IV	6	4	10	7	7	4	5	4	2	2	....	....	30	21	1.43
V	2	1	7	6	10	6	11	8	9	5	2	0	41	26	1.58
Totals	24	17	43	30	45	28	36	27	32	19	13	5	193	126	
Serv. per conc.	1.41		1.43		1.61		1.33		1.68		2.60		1.53		
Yolk-citrate diluent															
I	....	....	16	13	14	8	16	10	1	0	....	....	47	31	1.52
II	4	4	5	3	13	8	16	11	9	6	5	3	52	35	1.49
III	5	2	11	8	4	1	2	2	1	0	....	....	23	13	1.77
IV	2	0	15	10	5	4	4	4	4	1	....	....	30	19	1.58
V	1	1	8	7	8	5	5	3	4	4	....	....	26	20	1.30
Totals	12	7	55	41	44	26	43	30	19	11	5	3	178	118	
Serv. per conc.	1.71		1.34		1.69		1.43		1.73		1.67		1.51		

parently equal as preservatives of the motility of spermatozoa which were stored in them under standard conditions for two and four days.

3. The yolk-citrate diluent was superior to the yolk-phosphate diluent for the preservation of motility when semen was stored six days or more.

4. In actual insemination tests with semen stored up to 5 days no significant difference in fertility was found between the semen stored in the yolk-citrate and the yolk-phosphate diluents.

5. The results of the fertility studies tend to bear out the results of laboratory storage studies on the maintenance of motility under standard conditions.

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THE BACTERIOLOGY OF BULL SEMEN

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Practically all workers in the rapidly expanding field of artificial insemination stress the necessity of bacteriological control. It therefore seems desirable to know definitely what measure of bacteriological control is necessary or desirable in the collection, handling, and storage of semen. In addition, information on the types and numbers of bacteria present under varying conditions of collection and storage and their possible relation to infection in the female genital tract would be useful. Attention was earlier called to these facts by Salisbury, Willett and Gunsalus (14).

Most of the available information on the bacteria of the reproductive tract or semen of bulls deals either with the incidence and spread of disease, such as Bang's disease, or with possible causes of sterility of bulls with poor breeding records. Little attention has been given to the subject in relation to the preservation of semen and to artificial insemination.

Various methods have been used to obtain semen for bacteriological study. Webster (17), Gilman (4), and Williams and Kingsbury (19) recovered semen from the vaginas of cows immediately after normal service. The vaginas and sheaths having previously been douched and disinfected as suggested by Williams. Gilman, and Williams and Kingsbury reported finding micrococci, hemolytic and non-hemolytic streptococci, coliform organisms and *Brucella abortus*. They concluded that the genital tract and semen of normal bulls contained few, if any, bacteria, but that sterile bulls or those of diminished fertility usually contained large numbers. Webster reported that on culture normal semen yielded diphtheroids and micrococci, while bulls from herds in which enzootic sterility was present produced semen which contained, in addition, many alpha hemolytic streptococci. As suggested by Gilman, bacteriological studies on semen obtained by this technique are not entirely satisfactory due to possible contamination from the vagina.

Gilman (3) also studied the genital tracts of bulls shortly after slaughter and reported the presence of *Pseudomonas pyocyaneus* and unidentified rods in addition to the micrococci, streptococci, and coliform organisms. His findings also indicated that the genital tracts of normal healthy bulls were either free from bacteria or contained very low numbers, whereas impotent bulls harbored large numbers of organisms which were undoubtedly ejaculated with the semen. Gilman considered infection to be the greatest single

Received for publication June 12, 1941.

cause of functional and anatomical changes resulting in varying degrees of impotency.

Hatziolos (7) collected semen by use of the artificial vagina and attempted to obtain the semen as free from bacterial contamination as possible. The bacteria, which he found in every ejaculate, consisted mainly of coliform organisms, the proteus, and pseudomonas groups, cocci and spore forming rods.

That these types of bacteria can be responsible for metritis, cervicitis, sterility, or abortion in cows has been indicated by the observations of Lucet (9), Rosenow and Davis (12), Beaver, Boyd, and Fitch (1), Udall, Cushing and Fincher (16), and others.

Semen is a rather good medium for the growth of bacteria. Probably the first to report such an observation was Spallanzani (15) who in 1785 observed that the sperm from the terrestrial fetid toad soon became putrid. He attributed the diminishment of fertility of the sperm during storage to this putrefaction. Roemmele (11) noticed bacteria in semen stored at room temperature and at 9° C., and attributed the death of the sperm to the accumulation of the metabolic products of the bacteria. Hammond (6) observed bacterial growth in samples stored at 10° C., but found no greater incidence of infection in female rabbits inseminated with semen in which large numbers of bacteria had developed than with samples where fewer organism had grown due to a lower storage temperature. Krzyszkowsky and Pawlow (8) also mentioned that bacteria grew rapidly in semen, especially a small motile bacillus which formed a blue-green pigment (probably *Pseudomonas pyocyaneus*), though no definite data could be found to indicate whether bacteria have a direct detrimental effect on spermatozoa. Krzyszkowsky and Pawlow (8) thought that the products of metabolism of bacteria influenced the spermatozoon life. Gunn (5) on the other hand could detect no influence of bacteria on the survival of spermatozoa stored at low temperatures, (4° C.).

METHODS

The semen used in these studies was collected with the new-type artificial vagina (Salisbury and Willett (13)). The rubber liner of this device was sterilized by flushing with alcohol and drying before use. All glassware with which the semen came in contact was sterilized with dry heat. The mineral oil, petrolatum used as a lubricant, Milovanov's (10) SGC–2 diluent and phosphate buffers were sterilized by autoclaving.

The numbers of bacteria were determined by plate counts on blood agar containing 2 per cent sterile defibrinated horse blood. All plates were incubated 4 days at 37° C. This incubation period was selected in order to facilitate counting when slow growing diphtheroid organisms were present. The relative numbers of coliform bacteria were determined by serial dilutions in Durham tubes of glucose broth.

EXPERIMENTAL

Numbers and Predominating Types of Bacteria. The semen used in these studies was obtained from bulls in the University dairy herd and from bulls in the Central New York Artificial Breeders' Cooperative at Syracuse. The bulls at the University were used in the breeding program of the herd more or less frequently and bred the cows naturally. Since these bulls had access to exercise lots which often were muddy, their underlines and sheaths were frequently dirty. Unless the bulls at Syracuse had been brought into the herd only a short time before the samples were obtained they had not bred cows naturally for considerable periods of time, unless they had accidentally bred a cow in the breeding rack while the artificial vagina was being used. In addition, they were confined to stalls during the major portion of the day and were out-of-doors only while being exercised on a mechanical exerciser. As a result these bulls were clean about the underline and sheath.

TABLE 1

Number and kinds of bacteria found in fresh bull semen

Bull	Number ejaculates	Bacteria per cc. of semen (in thousands)		Predominating kinds
		Average	Range	
1	1	650	.....	Pseudomonas, *E. coli*
2	2	7	1– 13	Diphtheroids
4	1	20	.....	.....
6	1	15	.....	Diphtheroids
7	2	2	1– 2	" , Staphylococci
8	1	750	.....	Pseudomonas, *E. coli*
9	1	30	.....	Diphtheroids
21	1	90	.....	" , Bacilli
22	2	6	1– 12	"
23	1	1	.....	.....
24	1	4,300	.....	Diphtheroids, Pseudomonas, *E. coli*
26	1	50	.....	" , Staphylococci, *E. coli*
D	5	1,270	13– 4,900	"
E (1939)	6	4,600	290–22,000	" , (some hemolytic)
E (1940)	6	230	10– 750	" , Pseudomonas
F	2	120	5– 230	.....
H	4	6,500	10–20,000	Diphtheroids
I	2	260	130– 390	.....
J	1	1	.....	Pseudomonas
K	2	720	690– 750	.....

The data in table 1 indicate the numbers and predominating kinds of bacteria found in fresh ejaculates when no precautions were taken in cleaning the underline or sheath of the bulls prior to collection. Of the 12 bulls in the artificial breeding herd (represented by numbers in table 1), two-thirds gave plate counts of fifty thousand bacteria per cc. or less and only 3 gave plate counts of over one hundred thousand. One of these (number 24), which gave semen with over four million bacteria per cc., had not been

used previously for over three weeks, having just been added to the herd and not as yet put into use. All the other bulls had been used an average of once to twice per week before the samples were taken for examination. The other two samples with extremely high counts (numbers 1 and 8) contained large numbers of coliform and pseudomonas organisms as did number 24. All three of these bulls have since been removed from use in the artificial breeding circuits. None of the other samples contained as many as 100 coliform organisms per cc. The average of the logarithms of the plate count of these 15 samples was 22 thousand bacteria per cc. Of 28 semen samples from 7 bulls in the University herd (represented by letters in table 1), the majority contained from 100 thousand to several million bacteria per cc. Only four samples contained as few as ten thousand bacteria per cc. The average of the logarithms of the bacteria in the individual samples was 225 thousand per cc. whereas the arithmetic average of the counts was 2.3 millions. In comparison with the bulls in the artificial breeding circuit, these bulls produced semen containing 10 to 50 times as many bacteria. Whether this is due to frequency and kind of service (natural and artificial vagina) or other factors is not elucidated in this study.

The types of bacteria reported in these samples are only those which occurred in large numbers and not all types present. All the types reported by other workers as frequently found in semen have been found with the exception of streptococci. The organisms occurring most regularly in large numbers were diphtheroids. Hemolytic diphtheroid organisms were isolated from one bull (E) when one series of samples was taken, but they were not found a year later when a second series of samples were studied.

The number of bacteria present in these samples was higher than would have been expected from the reports of a number of workers who considered that semen from normal healthy bulls is almost free from bacteria. *Pseudomonas pyocyaneus* was isolated from five of the nineteen bulls studied. In this connection it should be noted that three of the four bulls dropped from breeding operation of the Central New York Artificial Breeders' Cooperative within the last six months were among the five in the semen of which pseudomonas organisms were present in large numbers. Of the other two bulls, one has since become sterile and the other has a record of 12.1 per cent conception from 91 inseminations by artificial breeding. Gilman (3) considered these organisms as among those of importance in genital infections.

Effect of Cleaning the Bull. In order to determine whether it would be possible to reduce the number of bacteria in semen collected with the artificial vagina, two bulls were cleaned, to the extent of thoroughly washing the underline with soap and water, clipping the hair from about the prepuce, and flushing the sheath with sterile distilled water. When bull (D) was treated in the above manner on alternate weeks, the first sample being col-

lected without cleaning the bull, the counts were as follows: 490, 3, 32, 3 in thousands. The reduction in count by the first cleaning was over a hundred fold and in the second case 10 fold, the count not having reached as high a value one week after cleaning as before any treatment. A second bull (I) which gave semen with a lower count without cleaning than D, did not show as great response to cleaning, the counts on consecutive weeks being 13, 1, 39, 6 in thousands. The bull was cleaned before the second and fourth collection. Here the reduction was about 10 fold, the counts being reduced to about the same number as those obtained when bull D was cleaned.

Preparation of Yolk-phosphate Diluent. It was found during the early course of the experiment that aseptic methods must be practiced in order to produce a yolk-phosphate diluent without undue contamination. To produce such a diluent, fresh eggs were obtained from a pullorum-free flock and immersed in normal NaOH or 70 per cent alcohol to sterilize the shell (Bryant and Sharp (2)). The eggs were then broken and the yolk removed, using sterile glassware, and mixed with sterile buffer. By using these precautions yolk-phosphate diluents with very low bacterial counts, could be consistently produced. Thus, of 21 samples of diluent so prepared, 17 had counts between 0 and 15 bacteria per cc., 2 of 150 and 200, and 2 had counts of 1,000,000 and 7,000,000. The last two samples mentioned may have been contaminated by the equipment or the buffer, for immediately after they occurred all the material was sterilized with the result that 12 consecutive samples were prepared during the following month with no count over 200. Four samples prepared with unsterilized buffer had counts ranging from 600,000 to 4,000,000, and two samples prepared with yolk from store eggs without sterilizing the shell or the buffer had counts of 24,000,000 and 166,000,000. As will be shown below, semen-yolk-phosphate mixtures containing such large numbers before storage often had counts in the billions after storage for several days. The yolk-phosphate diluent may be the important source of large numbers of bacteria in stored semen samples if care in preparation is not exercised.

Growth of Bacteria in Semen During Storage. The following studies were performed to investigate the growth of bacteria in undiluted or diluted semen during storage.

In one experiment 4 ejaculates were each divided into 3 parts and stored undiluted, in SGC–2 diluent and in yolk-phosphate diluent at 5° C. and 10° C. Samples from each treatment were examined after 4 and 8 days of storage. The data are summarized in table 2. It can be seen that the yolk-phosphate diluent was an excellent medium for bacterial growth as evidenced by the high count after storage for 8 days at 10° C. The bacteria grew least rapidly in the undiluted semen. As would be expected, there was considerably more bacterial growth at 10° C. than at 5° C. In fact, at the lower tem-

TABLE 2

Effect of storage temperature and diluent on bacterial growth (averages from 4 ejaculates)

Treatment	Bacteria per cc. in thousands after storage				
	5° C.			10° C.	
	0 days	4 days	8 days	4 days	8 days
Undiluted	190	420	400	430	740
Yolk-phosphate	200	250	520	600	12,000
SGC–2	100	130	80	310	2,600

perature there was not enough bacterial growth to effect changes in the medium. Therefore 5° C. or lower is preferable as a storage temperature in order to hold the bacterial growth to a minimum, especially since it has been shown by Willett (18) that storage at 5° C. preserves the viability of the sperm longer than higher storage temperatures.

During the course of Willett's study on the preservation of semen, other observations were made in regard to the growth of bacteria in semen-yolk-phosphate mixtures stored under different conditions. A comparison was made to determine if covering the diluted semen samples with a layer of mineral oil would reduce the growth of bacteria when they were stored for 4 and 8 days as had been indicated by certain preliminary observations. The data, from counts in 9 ejaculates, summarized in table 3 indicated that there was not enough difference between the two treatments to be of practical importance. Also, during the course of these bacteriological investigations several ejaculates were stored in the yolk-phosphate diluent in which the buffer was not sterilized. It was noted that the bacterial counts of such samples during storage reached numbers far exceeding those samples made with sterilized buffer. A summary of these counts is presented in table 3 for comparison with the counts of other uncontaminated samples. Since some workers in the field of artificial insemination recommended temperatures lower than 5° C. for semen storage, samples for 7 ejaculates were stored at both 5° and 1° C., and the bacterial counts compared after 4 and 10 days of storage. It can be seen (table 3) that there was no appreciable difference in numbers of bacteria after storage at the two temperatures.

During these studies of bacterial growth during storage, observations of spermatozoon motility and pH measurements were also made concurrently. The data show no definite relationship between the numbers of bacteria present and the motility of the spermatozoa or pH of the semen during storage. However, because we were largely successful in bacteriological control there were only a few samples in which enough bacteria developed to cause such changes.

The bacteria from bedding, manure, soil, unsterilized glassware, etc., would include *E. coli*, bacilli, and staphylococci. These are the types which

TABLE 3

Bacterial growth during storage in semen yolk-phosphate diluent

Sample pairs	Treatments	Bacteria per cc. (in thousands)					
		Before storage		After storage			
				4 days		8 days	
		Average	Range	Average	Range	Average	Range
No.							
9	Mineral oil, 5° C.	582	1–2,550	293	2– 2,100	426	6– 1,300
	No mineral oil, 5° C.	"	"	452	3– 3,550	533	4– 2,250
6	Diluent contaminated, 5° C.	2,490	170–5,500	165,000	100– 580,000	2,910,000	100– 7,150,000
	" " , 10° C.	"	"	464,000	100–2,040,000	12,000,000	175,000–28,500,000
7	5° C.	367	1–1,350	55	3– 105	341	4– 1,000
	1° C.	"	"	47	2– 145	352	2– 1,150

can be eliminated or materially reduced in number by the methods outlined above, and are the types which grow readily at storage temperatures. Therefore, if contamination by these types can be prevented, little bacterial growth will take place in the semen or semen-yolk-phosphate mixture during storage. On the other hand, it appears that the diphtheroids are always present in semen in variable numbers no matter what precautions are taken. However, this type does not grow at the usual low storage temperatures, and therefore, does not become numerous during long storage. In a number of samples pseudomonas organisms were present in large numbers. Since they grow at low temperatures, one would not be able to eliminate growth of these bacteria by using such temperatures.

Germicidal Action of Semen. In the storage experiments above reported it was observed that the number of bacteria decreased during storage in some cases. The question arose as to whether semen had a bactericidal action. Several experiments were performed to see if a bacteriacidal effect could be demonstrated at storage temperatures.

Semen from each ejaculate was divided into two parts. One part of the semen was stored untreated, while the other was inoculated with a culture of *Escherichia coli*. SGC–2 diluent inoculated with *E. coli* served as a control. The samples were stored between 5° and 7° C. without mineral oil in 25 cc. test tubes plugged with cotton. At the start of the storage period the counts were made every one-half or one hour, but the intervals were lengthened with the progress of the storage period. In three studies thus made at these low temperatures, there was no evidence of any germicidal action.

During these experiments frequent observations of spermatozoon motility were also made with rather startling results. It was found in all three experiments that the motility of the spermatozoa in the inoculated samples was markedly superior to those in the untreated semen when diluted and examined after storage. In one experiment a sample of semen was inoculated with coli killed by heating to compare with the other treatments. The spermatozoa in the sample showed no greater motility than the untreated semen.

SUMMARY

1. The bacterial count on 43 ejaculates collected from 19 bulls by means of an artificial vagina ranged from 1,000 to 22,000,000 per cc.

2. It was found that by douching the sheath and washing the underline, if the bull was dirty, the number of bacteria in semen could be markedly reduced.

3. Almost sterile yolk-phosphate diluent was consistently produced when fresh eggs from healthy hens were used and when aseptic methods were employed in the preparation of the diluent. Under other conditions the

diluent may be responsible for the addition of large numbers of bacteria to semen samples.

4. Bacterial growth during storage was held at a minimum by storing at 5° C. or lower.

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PSEUDOMONAS PUTREFACIENS IN DAIRY PLANT EQUIPMENT*

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In outbreaks of putrid butter the sources of the causative organisms undoubtedly vary. Some outbreaks have been traced to water used to wash the butter or equipment (1, 3), while in other instances water has not been involved. *Pseudomonas putrefaciens* commonly is responsible for the putrid defect in salted butter (1, 2), at least in certain areas. It is sensitive to heat, being killed in 2 minutes at 61.7° C. (4), so that it probably never survives pasteurization. A source other than water which could contribute the organism to pasteurized cream or to butter is dairy plant equipment. Attempts to isolate *Ps. putrefaciens* from such equipment are reported herein.

METHODS

Frequently, bacteriological examinations of dairy plant equipment have involved rinsing with sterile water and then culturing the water. In the case of churns this is unsatisfactory because many organisms are between staves, at junctions of ends and staves, around bolts, etc., from which they are dislodged only when there is considerable strain on the churn, for example, when the butter breaks or when it is worked. In these locations the organisms are protected during the attempts to sterilize the churns, and their multiplication continues to supply organisms for the contamination of one lot of butter after another. Much the same situation probably exists with certain other equipment, especially leaky vats.

In the isolation studies reported herein such materials as curd and wood from various points in the equipment were smeared directly, and also after enrichment in litmus milk at 3° C. for varying periods, on a special medium (4) consisting of: gelatin 4 per cent, proteose peptone 2 per cent, dipotassium phosphate 0.1 per cent, ferric ammonium citrate 0.05 per cent, agar 1.5 per cent and water to make 100.0 per cent. On this medium *Ps. putrefaciens* grows relatively well at 21° C. and gives colonies with a rather characteristic appearance. Colonies suggesting *Ps. putrefaciens* were purified and enough of the characters studied to determine whether the organisms belonged to this species.

RESULTS

In some instances *Ps. putrefaciens* was obtained from dairy plant equipment and in other instances isolation attempts failed. Examples of both successful and unsuccessful attempts are as follows:

Received for publication June 16, 1941.

* Journal Paper No. J-228 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 119.

Example 1. In a plant making butter that kept satisfactorily in normal marketing channels, an occasional churning became putrid in 5 to 7 days in keeping quality tests at 21° C. Pasteurization of the cream appeared to be efficient, and the wash water was chlorinated. Materials were collected from various parts of two of the several churns in the plant by loosening the shelves, removing bolts, etc.

With churn 1, *Ps. putrefaciens* was recovered by direct smears from between the end of a shelf and the iron cover plate and from wood near a bolt; it was recovered by enrichment from three of four points near bolts; and it was not recovered from five points, including the buttermilk drain, around the sight glass, under a bolt head and on the brace (two points) under a shelf. With churn 2, the organism was recovered directly from the end of the shelf and from under the shelf but not from two other points near the shelf; enrichment did not detect additional positive samples.

Microscopic examinations of the original material indicated that micrococci, gram-positive rods, gram-negative rods, yeasts and molds were present, often in large numbers; these general types of organisms also were evident on the plates.

Example 2. Some months after the collection of the samples referred to in example 1, the spoilage of butter in the keeping quality tests no longer occurred in the plant. Because of its general condition it was necessary to replace an end in one of the churns and during the operation six samples were obtained from around bolts, under the sight glass and at the junction of shelves and ends. None of the samples yielded *Ps. putrefaciens*, either directly or by enrichment. All the plates were heavily overgrown with various microorganisms.

Example 3. A coil vat in a milk plant was discarded because of leaks which permitted liquid to soak into the insulation. Within 24 hours of the last use of the vat, holes were cut through the outer wall and samples of the insulation collected. Near the leaks the insulation was soaked with water and had a very objectionable putrid odor, while at some distance from the leaks it was moist and the odor was somewhat putrid.

Ps. putrefaciens was isolated by direct smears from four of the seven samples but was not obtained by enrichment from any of them. Three of the four positive samples were from portions of the insulation that were very wet, while one was from a portion that was only moist.

After the insulation had dried rather completely over a period of 5 weeks, eight additional samples were collected. Cultures of these, both without and with enrichment, failed to show *Ps. putrefaciens*, the plates being largely overgrown with aerobic spore-forming bacteria.

Example 4. A churn barrel was taken out of a plant because the general condition, particularly of the roll, made repairs inadvisable. The plant had not experienced the putrid defect; however, the butter was consumed

quickly and keeping quality tests were not being run. Since the barrel was to be used for holding water it could not be dismantled and only the roll and one shelf were removed. Material was collected from one end of the roll, from one end of the shelf, from around the shelf support and from around bolt heads.

Ps. putrefaciens was not detected on plates smeared with the different samples, either directly or after enrichment. However, the plates were heavily overgrown with various microorganisms, including micrococci, spore-forming bacteria, gram-negative rods, yeasts and molds.

Example 5. A plant experiencing a serious outbreak of putrid butter quickly brought the difficulty under rather complete control as far as spoilage in commercial channels was concerned. However, many churnings continued to show spoilage after 5 or 6 days in keeping quality tests at 21° C. Various procedures, such as chlorination of the water, unusually high pasteurization of the cream, etc., failed to prevent the spoilage in the keeping quality tests. The barrel of the older of the two churns in the plant was replaced and about 15 inches of one end of the barrel was cut off and sent to the laboratory. Over a period of several days, 67 samples were collected from various parts of the end which was completely broken up in the process.

Ps. putrefaciens was recovered directly from 13 of the samples; 8 of these were from points at the junction of staves and the end of the churn, 2 from between boards in the end of the churn, 2 from under the sight glass and 1 from wood near a bolt. After enrichment the organism was obtained from 3 additional samples, 2 being from points at the junction of staves and the end of the churn and 1 from between boards in the end of the churn. In some instances *Ps. putrefaciens* made up a rather large percentage of the colonies on the plates smeared directly with a sample. The flora of many of the plates also included micrococci, spore-forming bacteria, gram-negative rods and occasionally some yeasts and molds.

DISCUSSION

The isolation of *Ps. putrefaciens* from various points in churns and from the insulation of a leaky vat indicates that equipment may be a source of the organism in dairy products. Evidently *Ps. putrefaciens* survives the cleaning and heating of equipment due to protection afforded in crevices, cracks and joints. The churns from which *Ps. putrefaciens* was isolated were reported to have received thorough and regular cleaning.

The fact that plates smeared with material from churns not yielding *Ps. putrefaciens* often were heavily seeded with other species, many of which were not heat resistant, is additional evidence that conditions in certain churns may be favorable for *Ps. putrefaciens.* It also suggests that equipment may yield other organisms capable of causing defects in dairy products.

The flora of certain plates smeared with material from churns definitely

resembled the flora of plates prepared from putrid butter. Such plates, regardless of whether or not *Ps. putrefaciens* is present, usually contain micrococci, spore-forming bacteria, gram-negative rods and often yeasts and molds. This suggests that with defective and highly contaminated butter much of the contamination may originate in the churn.

SUMMARY

Ps. putrefaciens was isolated from churns and from the insulation of a leaky vat. At certain points the organism was present in considerable numbers. In addition to *Ps. putrefaciens*, micrococci, spore-forming bacteria, gram-negative rods and often yeasts and molds were present.

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OXIDIZED FLAVOR IN MILK. IX. THE EFFECT OF THE QUALITY OF HAY AND EARLY STAGE OF LACTATION ON THE CAROTENE CONTENT OF BUTTERFAT AND ON THE ASCORBIC ACID CONTENT OF THE MILK AND THEIR RELATIONSHIP TO THE DEVELOPMENT OF METAL-INDUCED OXIDIZED FLAVOR*

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One of the earliest workers to report a seasonal variation in oxidized flavor was Mattick (11) who in 1927 reported that oiliness in milk appeared in autumn, winter and spring, but never in the summer. Kende (10) recognized this fact and as a result of his work concluded that green feed contained some substance or substances which when fed to the cow protected the milk against the off-flavor. Since that time numerous workers have observed the difference in the susceptibility of winter and summer milk to oxidized flavor. Stebnitz and Sommer (15) found that when cows received grass as part of their ration, the butterfat became less saturated and more susceptible to oxidation. However, it appeared that protective substances in the milk prevented the development of oxidized flavor. Likewise, it is generally agreed that green feeds yield a more stable flavored milk as compared to milk produced on dry feed.

The nature of these inhibiting substances carried in green feed has been the object of extensive investigations. Anderson and co-workers (1, 2) were some of the earliest investigators to show the effect of carotene on the milk flavor. Their work showed that carrots or machine-cured alfalfa fed to cows would eliminate oxidized flavor in their milk. This they correlated with the carotene content of the feed. Anderson, Hardenbergh and Wilson (2) obtained far more effective results in the elimination of oxidized flavor by feeding 8 pounds of carrots in the daily ration than by feeding a cod-liver oil concentrate containing 500,000 U.S.P. units of vitamin A. Whitnah and co-workers (17, 18) and Beck, Whitnah, and Martin (4) report that a carotene supplement quickly corrected the tendency for oxidized flavor to develop spontaneously. In addition they found oxidized flavor more prevalent in milks that were below the breed average in fat color intensity. There were, however, samples low in color which did not develop oxidized flavor. Likewise, it was noted that the color of the fat was not increased for some time

Received for publication June 16, 1941.

* Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 267.

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after the flavor was improved. These workers (18) report that low-carotene intake levels were not the only factor determining the tendency for milk to develop oxidized flavor as shown by the fact that the five lowest carotene intakes per kilogram of body weight among cows producing milk that kept a good flavor were below the three highest intakes among these cows producing milks which developed oxidized flavor.

Brown, Vanlandingham, and Weakley (6) found that a carotene supplement added to the ration rendered the milk less susceptible to metal-induced oxidized flavor. Likewise, the supplementing of a low-carotene ration with ascorbic acid produced similar results. Garrett, Tucker, and Button (9) found a positive correlation between color and flavor and between ascorbic acid and flavor. They concluded that high carotene and high ascorbic acid are coincidental to and help to preserve good flavor in milk. Garrett, Arnold, and Hartman (7) reported that alfalfa silage is almost equal to spring pasture in putting yellow color into milk and is equal to or better than pasture in producing milk of fine flavor and high resistance toward the development of oxidized flavor.

Brown, Thurston, and Dustman (5) found that feeding of one quart per animal per day of either tomato or lemon juice to cows on dry feed reduced the susceptibility of the milk to oxidized flavor. They attributed this effect to ascorbic acid in the feed and observed a similar tendency when pure crystalline ascorbic acid was fed at the rate of $\frac{1}{2}$ gram daily. Nelson and Dahle (12) added orange and tomato juice directly to the milk and observed a slightly greater protective effect than could be accounted for on the basis of their ascorbic acid values.

Garrett, Tucker, and Button (9) obtained information based on the average flavor scores which showed that for a decrease in ascorbic acid there was also a corresponding decrease in flavor score. The apparent critical point of the relationship of ascorbic acid to good flavor was found to lie between 15 and 18 mg. of ascorbic acid per liter of milk. Garrett, Arnold, and Hartman (7) reported that feeding grass silage had a greater stabilizing effect on ascorbic acid in milk than corn silage or beet pulp. This stabilizing effect tended toward milk of better flavor.

It has been shown that feeding carrots or machine-cured alfalfa hay will render milk non-susceptible to oxidized flavor and that both carotene and ascorbic acid in the feed play a role in the susceptibility of the milk to the flavor. Since the carotene in the butterfat is affected by the carotene in the feed, and since good quality hay is known to contain relatively large amounts of carotene, it appears that there should be a relationship between the quality of hay and the susceptibility of the milk to oxidized flavor. Since many producers who have difficulty with oxidized flavor purchase hay, it seemed desirable to know if the feeding of very high quality alfalfa hay would render the milk non-susceptible to metal-induced oxidized flavor. Accordingly, the following experiment was planned and conducted.

EXPERIMENTAL

For use in this experiment, eight Jersey cows, whose milks were susceptible to oxidized flavor when contaminated with copper, were selected and placed on a ration low in carotene. The feed consisted of a grain mixture of 100 lb. ground oats, 100 lb. wheat bran, 15 lb. cottonseed meal, 3 lb. salt, and 2 lb. of steamed bone meal, with different quality alfalfa hay and beet pulp as the roughage.

Three quarts of milk were collected from each cow at the morning milking on the first three consecutive days each week, and carotene, ascorbic acid, and flavor determinations were made. The ascorbic acid was determined on the individual samples of raw milk, as soon as possible after milking, by titrating as suggested by Sharp (16). The remaining milk was pasteurized in bottles. Following pasteurization and cooling, four one-half pint samples were prepared containing none, 0.5, 1.0, and 1.5 parts per million, respectively, of copper from a copper sulphate solution. These samples were then stored in ice water for three days, after which they were scored for flavor by at least three judges familiar with oxidized flavor. The remainder of the milk was prepared for carotene analysis by gravity separation of the cream followed by churning. Before churning, the cream from each of the three days was composited so as to make one churning and one analysis for carotene per cow per week. The butter thus obtained was melted and centrifugalized in Hart's casein tubes in an electrically heated centrifuge for 15 minutes, after which the clear, liquid butter oil was decanted into a clean, dry jar. The carotene analyses were made according to the method of Baumann and Steenbok (3) modified by Rogers and associates (14). After a 5½-week preliminary period on the regular herd ration the animals were changed to the experimental depletion ration for a period of four weeks. This ration consisted of the grain mixture already described, and eight pounds of brown alfalfa hay which still retained its leaves, together with 12 pounds of dry beet pulp.

At the end of the depletion period the animals were divided into 2 groups which were approximately equal in intensity of oxidized flavor. One group was continued on the brown hay roughage while the other group was changed to bright green, leafy, alfalfa hay. The hay given both groups was increased from 8 to 12 pounds per animal per day. Special care was taken to select hay of equal leafiness in both types of hay. The brown hay had an average carotene content of 5.8 mg. of carotene per kilogram whereas the green hay had an average carotene content of 43.0 mg. of carotene per kilogram. After two weeks on this ration the cows that were receiving the bright green hay were given hay *ad lib.* for three weeks and then were supplemented with 2 pounds of alfalfa leaf meal (carotene content 4.9 mg. of carotene per kilogram) daily. The purpose of this supplement was to increase the amount of alfalfa and carotene in the ration. Unfortunately, the amount of caro-

tene in the leaf meal was lower than expected and therefore the carotene supplement was not as great as was intended. However, there was a marked reduction in the intensity of the oxidized flavor of the milk.

After the three-week period in which the cows received leaf meal supplement, there was a readjustment period of three weeks followed by pasture for one week, after which time the experiment was discontinued. The cows

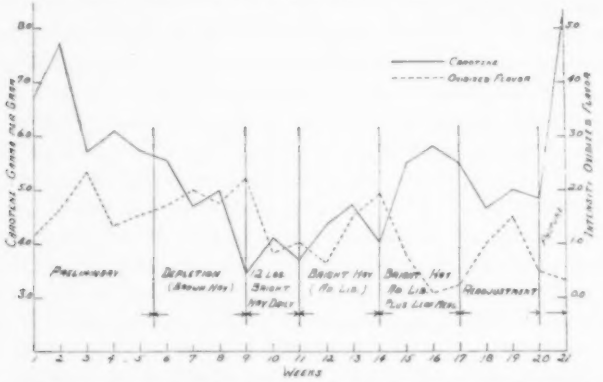

FIG. 1. The relationship between carotene and metal-induced oxidized flavor in the milk produced by cows on bright, green, alfalfa hay.

that received the brown hay were continued on the same basis except that they received no alfalfa leaf meal supplement. These animals were on pasture one week before the experiment was discontinued. The results of this experiment are shown graphically in figures 1 and 2. Figure 1 gives the average of the results obtained with the group on bright green hay while figure 2 gives the average results obtained on brown hay during the same periods.

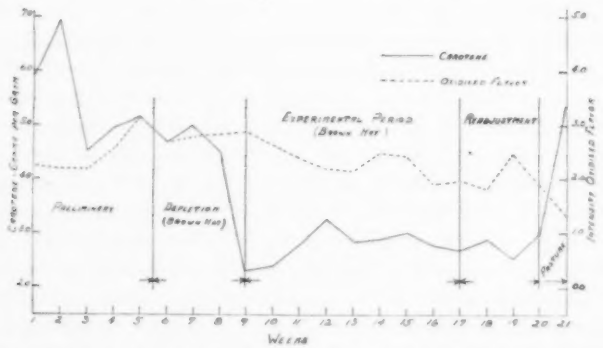

FIG. 2. The relationship between carotene and metal-induced oxidized flavor in milk produced by cows on a low-carotene ration receiving brown hay.

The results shown in figure 1 indicate that there may be a relationship between the carotene in the butterfat and the intensity of oxidized flavor developed, but this relationship is not very clear. During the first nine weeks there was a great reduction in the carotene of the butterfat, but there was no significant increase in the susceptibility of the milk to oxidized flavor. When the ration was supplemented with alfalfa leaf meal even though relatively low in carotene *per se*, there was an increase in the carotene of the butterfat and a corresponding decrease in the intensity of the oxidized flavor until it was almost eliminated. It may be observed, however, that the carotene in the butterfat, during the period when the ration was supplemented with alfalfa meal and practically devoid of oxidized flavor, was not as high as at the beginning of the experiment, when the oxidized flavor was relatively strong.

Figure 2 shows the relationship between the carotene content of the fat and the intensity of oxidized flavor of milk produced on brown hay. Here there seems to be an absolute lack of relationship, since the carotene content of the milk was greatly reduced and maintained at a low level for some time. It might be expected that the intensity of the oxidized flavor would be increased and that possibly some spontaneous oxidized flavor development might occur. However, examination of the data reveals that, contrary to the expected results, we find if anything a slight reduction in the intensity of the oxidized flavor developed.

There was no apparent relationship between the ascorbic acid content of the milk and the susceptibility of the milk as influenced by the quality of hay.

STAGE OF LACTATION

While the experiment on the quality of hay was in progress it was noticed that certain animals which had recently calved were producing milk relatively high in carotene but nevertheless with a strong oxidized flavor. It was decided to study the effect of early stage of lactation on the development of this flavor. Accordingly, samples were taken from nineteen cows which had just freshened on the herd ration. Carotene, ascorbic acid, and metal-induced oxidized flavor development in their milks were studied. All determinations were made in the same manner as in the previous experiment. The results obtained from the 19 cows studied are shown in figure 3. Examination of these data reveals that there was a marked decrease in the carotene of the butterfat for the first three weeks of lactation, after which it remained fairly constant for the next eight weeks. The intensity of the oxidized flavor developed followed a very similar curve and almost paralleled the carotene curve. In contrast to the results of Rasmussen *et al.* (13), it was found that the ascorbic acid content at the beginning of lactation was unusually low and increased gradually until it reached a maximum level at about eight weeks following parturition. These results verify the work of Whitnah and Rid-

dell (19), who found an average increase of 10 per cent in vitamin C concentration from the first to the second month of lactation. In this experiment there appeared to be an inverse relationship between the amount of ascorbic acid in the milk and the intensity of the metal-induced oxidized flavor.

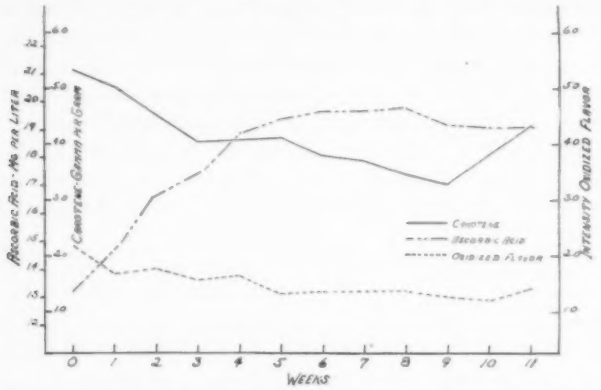

FIG. 3. The relationship between the carotene content of the butterfat, the ascorbic acid content of the milk, and the intensity of metal-induced oxidized flavor, as shown during the early stages of lactation.

Since there was no apparent relationship between the ascorbic acid content of the milk and the intensity of the oxidized flavor depending upon the quality of the hay, it was decided to arrange all values obtained in groups based on the intensity of the oxidized flavor. Figure 4 shows the relationship of ascorbic acid to intensity of oxidized flavor as shown by 580 observa-

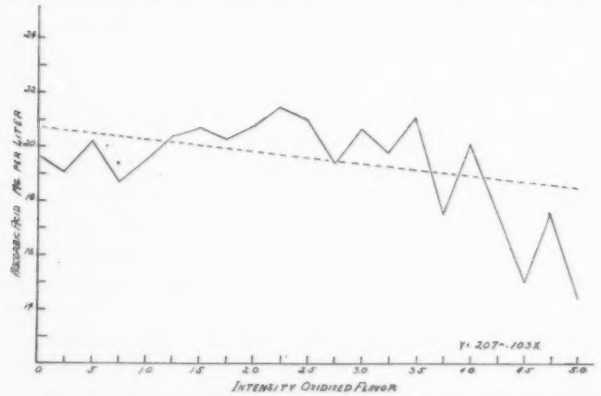

FIG. 4. The relationship between the ascorbic acid content of the milk and the intensity of metal-induced oxidized flavor.

tions. The broken line is the "line of least squares" as calculated from the individual observations. Although the slant of the line is not steep it appears that there is a tendency for milk which develops a strong degree of oxidized flavor to have a lower original ascorbic acid content. This compares favorably with results in the literature previously mentioned (9).

Carotene likewise has been claimed to be a major factor in the susceptibility of milk to oxidized flavor. It seemed desirable to plot the carotene content against the intensity of the oxidized flavor in the same manner. The results of this study are shown in figure 5 which represents the data from

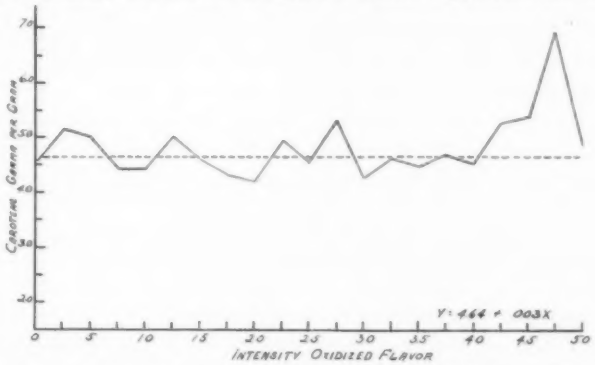

FIG. 5. The relationship between the carotene content of the butterfat of herd milk and the intensity of metal-induced oxidized flavor.

555 observations. The line of least squares as plotted through these data reveals the lack of relationship between the two variables. Since it is common knowledge that there are breed differences in the ability of cows to

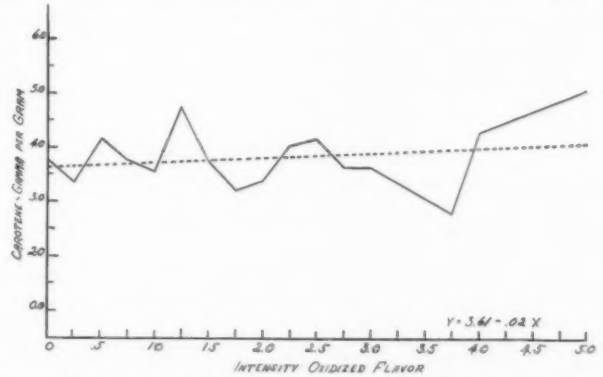

FIG. 6. The relationship between the carotene content of the butterfat of Ayrshire milk and the intensity of metal-induced oxidized flavor.

transfer carotene to the milk it was thought that this might have obscured any relationship which might have existed. Accordingly the breeds were divided into separate groups and plotted separately. These results are shown in figures 6, 7, and 8.

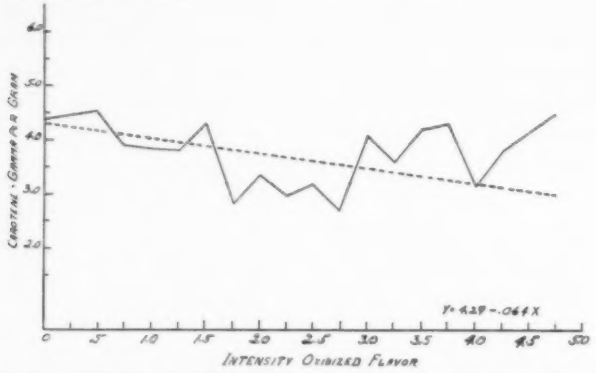

FIG. 7. The relationship between the carotene content of the butterfat of Holstein milk and the intensity of metal-induced oxidized flavor.

Figure 6 gives the results obtained for 6 Ayrshire cows with a total of 121 observations. The line of least squares shows, if anything, a direct relationship between the intensity of the oxidized flavor and the carotene content of the milk. However, in figure 7, the data obtained from 8 Holstein cows with 132 observations give a line of least squares slanting strongly in the opposite direction, indicating an inverse relationship between carotene and oxidized flavor. Figure 8, which represents 14 Jersey cows with 302 observations, is just about a median line between the two extremes. In a

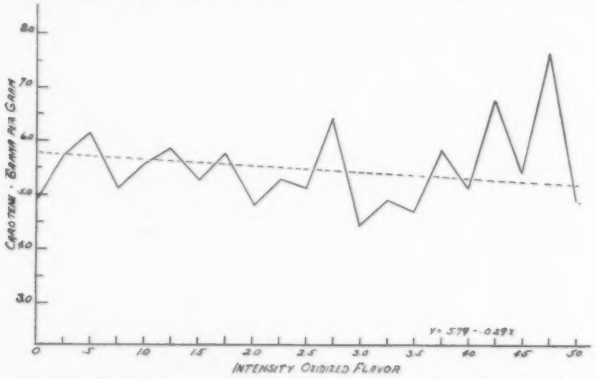

FIG. 8. The relationship between the carotene content of the butterfat of Jersey milk and the intensity of metal-induced oxidized flavor.

random distribution we would expect as many observations on one side as on the other of a horizontal line. This is the condition which exists in these data, indicating the lack of relationship between the carotene content of the milk produced on dry feed and the intensity of the metal-induced oxidized flavor.

Since all these observations were made from cows on dry feed, it is entirely possible that different results might be obtained from cows on pasture.

DISCUSSION

The results of the experiment on the quality of alfalfa hay fed to cows producing milk susceptible to oxidized flavor show that it is possible to reduce greatly or entirely eliminate the susceptibility of the milk to oxidized flavor development by feeding excellent quality alfalfa hay. The feeding of brown, leafy alfalfa hay to cows caused an appreciable decrease of the amount of carotene in the milk but did not result in an increase in the intensity of the metal-induced oxidized flavor of the milk. A summarization of these data together with those obtained from a number of cows on a normal herd ration suggests that the carotene of the milk may not be the substance responsible for the reduction in the susceptibility of the milk to oxidized flavor but that some other substance or substances associated with it in bright green hay or alfalfa leaf meal may be the important factor or factors.

Likewise, these data suggest that the ascorbic acid content of the milk may play a minor role in the susceptibility of the milk to oxidized flavor. The result of the study of nineteen cows from the time when their milk became ready for human consumption until they had been in production for three months suggests that the ascorbic acid content of the milk is low at the time of parturition and increases for a period of about seven or eight weeks after which it remains fairly constant for that particular animal. This increase apparently resulted in a slight decrease in the intensity of the oxidized flavor developed during the same period.

SUMMARY AND CONCLUSIONS

1. The feeding of high quality alfalfa hay together with alfalfa leaf meal greatly reduced or eliminated the tendency for metal-induced oxidized flavor to develop.

2. The feeding of brown, leafy alfalfa hay resulted in a decreased carotene content in the milk but did not increase the intensity of the oxidized flavor.

3. The carotene content of the milk fat at the beginning of lactation appears to be high and decreases until it reaches a normal level a few weeks after parturition.

4. The ascorbic acid content of milk at the start of lactation is usually low and increases gradually until it reaches a maximum level at about seven or eight weeks following parturition.

5. From the results obtained it appears that ascorbic acid in the milk plays a minor role in the susceptibility of the milk to metal-induced oxidized flavor.

6. The results of this study indicate that the amount of carotene in the butter fat may not be the substance responsible for the reduction in susceptibility of milk to oxidized flavor. It appears that some substance or substances associated with it probably has a greater effect than the carotene itself.

ACKNOWLEDGMENT

The authors are indebted to Mr. L. J. Manus, Department of Dairy Husbandry, and Mr. C. G. Cook, Superintendent of the University Creamery, for assistance in scoring the experimental samples of milk.

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A DOUBLE CHANGE-OVER DESIGN FOR DAIRY CATTLE FEEDING EXPERIMENTS*

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INTRODUCTION

During the winter of 1939–40, a feeding trial was carried out by the Iowa Agricultural Experiment Station with eighteen Holstein cows, on three planes of feeding. The three rations tested, and hereinafter designated as A, B and C, respectively, were as follows:

A. Roughage alone (alfalfa hay and corn silage).

B. Limited grain (alfalfa hay, corn silage and grain fed at the rate of 1 pound for each 7 pounds of milk produced).

C. Full grain (alfalfa hay, corn silage and grain fed at the rate of 1 pound for each 3.5 pounds of milk produced).

The grain mixture consisted of the following constituents in parts by weight: corn, 4; oats, 4; cracked soybeans, 1; bone meal, 0.25; and salt, 0.15.

The experiment was confined to a single lactation, and was of the short-time switch-over type, each cow receiving each ration for a period of six weeks. The layout of the experiment was designed so as to avoid as far as possible the difficulties that commonly arise in interpreting the results of switch-over trials, and to permit tests of significance of the differences between the effects of the rations. It is hoped that a brief description of the layout and of the statistical analysis may be of interest to those engaged in dairy work.

DESCRIPTION OF THE DESIGN

In the ordinary group trial, where each cow receives only a single ration, the average milk-yield obtained for a given ration depends on the yielding ability of the cows receiving the ration. Since cows are highly variable in this respect, the experimental error from this source is often large, though it may be reduced by skillful grouping of the cows. The switch-over trial represents an attempt to eliminate this source of variation entirely from the comparisons between rations, by feeding every cow in turn on all rations. However, in designing switch-over trials, other sources of experimental error must be taken into account if the maximum gain in accuracy is to be realized. One arises from the characteristic drop in milk yields towards the end of the lactation period. Thus if a cow receives rations A, B and C in succession, her total milk yields under the three rations are scarcely comparable, since A was tested during the most productive period and C during the least. In

Received for publication June 16, 1941.

* Journal Paper No. J-199 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project Numbers 416 and 514.

most switch-over trials whose results we have examined, this drop in yield is a preponderating factor, the yields falling even when the poorest ration is given first and the best ration last.

This difficulty can be largely overcome by designing the experiment so that in any experimental period one-third of the cows are receiving each ration. For instance, one-third of the cows might receive the sequence ABC, one-third the sequence BCA, and one-third the sequence CAB. With this arrangement the three experimental periods are equally represented in the average milk yield for any ration. However, the effects of the lactation curve are not eliminated entirely from the experimental error of these averages unless the natural rate of fall in yield from period to period is the same for the three groups of cows. For example, if the sequence ABC is given to a group of cows whose lactation curves show high yields in the first period, thereafter dropping rapidly, whereas the other two sequences are given to cows whose lactation curves have a much smaller rate of fall, ration A is favored relatively to B and C when the average yields over all three groups are calculated. Thus the division of the available cows into three groups to receive the three sequences should not be made simply at random; instead, the object should be to obtain groups which are as similar as possible in the rates of fall of their lactation curves.

In the present experiment, the eighteen cows were first divided into six groups of three on the basis of expected yielding ability, the three cows within each group being chosen as similar as possible. The three sequences were then allotted at random to the three cows of each group. The reasoning was that cows with the same yielding ability would be likely to show a similar rate of fall in yields. This assumption appeared to be justified by the results of the trial in which this grouping was used, the total rate of fall per group decreasing markedly from the high-yielding to the low-yielding groups, as shown in table 1. In this table, as in subsequent tables, all the milk yields were converted to four per cent, fat-corrected milk (2).

The decreases in yield from period I to period II and from period II to period III were almost twice as great for the high-yielding groups as for the low-yielding groups.

TABLE 1

Total yield and rate of fall in yield of fat-corrected milk

	Groups* of three cows				
	1	2	4	5	6
Total milk yield (lbs.)	14,351	14,168	12,735	11,391	10,603
Decrease in yield (lbs.)					
Period I–II	868	889	385	521	472
Period II–III	1,019	923	598	506	495

* Group 3 omitted because one cow became sick and had to be rejected.

With this design, each group of three cows constitutes an independent experiment, of a type known as the 3×3 Latin square (fig. 1) (1).

Period	Cow		
	1	2	3
I	A	B	C
II	B	C	A
III	C	A	B

FIG. 1. The 3×3 Latin Square Design (No. 1).

A valid estimate of the experimental error may be obtained by an analysis of variance, as follows:

	Degrees of freedom
Between cows	2
Between periods	2
Between rations	2
Error	2
Total	8

Differences between cows and between periods are not included in the estimate of error, since by the nature of the design they are excluded from the true errors of the ration means. Each of the six groups provides a separate analysis of variance of the above type. On combining the six analyses, the following subdivision of degrees of freedom is obtained for the whole experiment:

	Degrees of freedom
Between groups	5
Between cows within groups	12
Between periods within groups	12
Between rations	2
Interaction of rations and groups	10
Error	12
Total	53

The 12 degrees of freedom for *between cows, between periods* and *error* are the totals of the corresponding terms in the individual groups. A similar set of 12 degrees of freedom is obtained for *between rations.* This may be divided into two parts: 2 degrees of freedom representing the average differences between rations over the whole experiment, and 10 degrees of freedom representing variations from group to group in the differences between the rations. If the differences between the rations are the same in all groups (apart from experimental errors), the mean square for this term should be no larger than the error mean square, and may legitimately be included in the error. However, the term should be calculated separately to test this point, particularly where the groups differ widely in yielding ability or in other respects, *e.g.*, breed. The remaining 5 degrees of freedom, corresponding to differences between the group totals, are not included in the individual analyses, being given here for completeness.

For reasons to be explained later, three of the groups were assigned to the Latin square given above, and the remaining three to the complementary square as shown in figure 2. This feature introduces no change in the method of analysis.

A B C
C A B
B C A

FIG. 2. The 3 × 3 Latin Square (No. 2).

ANALYSIS OF VARIANCE OF THE TOTAL DIGESTIBLE NUTRIENT CONSUMPTION

The consumption of digestible nutrients per cow for each period is given in table 2. Each of the groups in this table was analyzed separately and the degrees of freedom, as well as the sums of squares, were combined (table 3), according to the procedure previously described. The data on group 3 were omitted from table 2 due to the loss of one of the cows in it, thus causing a loss of nine degrees of freedom in the combined analysis.

TABLE 2

Individual total digestible nutrient consumption units: lbs. per cow per period (total of six weeks)

Group 1								Group 2							
Period	Cow 1		Cow 2		Cow 3		Period total	Cow 4		Cow 5		Cow 6		Period total
I	A	608	B	885	C	940	2433	A	527	B	696	C	989	2212
II	B	715	C	1087	A	766	2568	C	883	A	635	B	899	2417
III	C	844	A	711	B	832	2387	B	785	C	901	A	657	2343
Total		2167		2683		2538	7388		2195		2232		2545	6972

Group 3								Group 4							
	Cow 7		Cow 8		Cow 9			Cow 10		Cow 11		Cow 12		Period total
This group omitted because cow No. 7 was removed from the experiment in Period I, owing to kidney cancer.								A	472	B	734	C	897	2103
								C	819	A	644	B	766	2229
								B	778	C	953	A	706	2437
									2069		2331		2369	6769

Group 5								Group 6							
Period	Cow 13		Cow 14		Cow 15		Period total	Cow 16		Cow 17		Cow 18		Period total
I	A	586	B	635	C	805	2026	A	489	B	593	C	788	1870
II	B	723	C	799	A	542	2064	C	730	A	536	B	695	1961
III	C	892	A	595	B	681	2168	B	674	C	758	A	609	2041
Total		2201		2029		2028	6258		1893		1887		2092	5872

A = roughage, B = limited grain, C = full grain.

TABLE 3

Analysis of variance (3) of t.d.n. consumption units: total consumption per cow per period, in lbs.

	Degrees of freedom		Sums of squares		Mean squares	
1. Between groups	4		157,936		39,484†	
2. Between cows within groups	10		105,336		10,534†	
3. Between periods within groups	10		40,534		4,053*	
4. Between rations	2		533,869		266,934†	
5. Ration × group interactions	8	18	12,021	26,121	1,503	1,451
6. Error	10		14,100		1,410	
7. Total	44		863,796			

* Significant.
† Highly significant.

Since the analysis of variance in table 3 shows the "ration × group interaction" to be insignificant, this term was included in the experimental error. The mean square of the ration effects (266,948) is significantly greater than the error mean square (1450), signifying that the consumption of nutrients was appreciably increased by the addition of grain to the ration. The F-test (3) reveals that the differences between groups are also highly significant, the mean square for that term being 39,490 as compared with only 1450 for the error. This shows that the arrangement of cows into outcome groups was well justified.

TABLE 4

Digestible nutrient consumption-totals and means units: lbs. for six weeks periods

Rations	Ration totals (15 cows)	Ration means	Standard error
A	9,083	605.5	
B	11,091	739.4	± 9.83
C	13,085	872.3	
Grand total =	33,259	General mean = 739.1	

Table 4 shows the mean digestible nutrient consumption for each ration, and the standard error per cow. The standard error, $\sqrt{1451} = 38.08$ is 5.15 per cent of the general mean, 739.1. The standard error for the mean of 15 cows is $38.08/\sqrt{15} = 9.83$ pounds, or only 1.33 per cent of the mean. The relative performance of the groups was in the order anticipated, all differences between pairs of rations being highly significant.

ADJUSTMENTS FOR CARRY-OVER EFFECTS

In the case of total nutrient consumption the amount eaten in one period did not appear to be influenced by the ration given in the previous period. However, with other factors, particularly total milk yield, a carry-over effect of the ration given in the previous period may be anticipated, owing to the shortness of the change-over period from one ration to another.

If such residual effects are present, simple averages do not give unbiased estimates of the effects of the rations. For instance, with the layout shown in figure 1, ration A is preceded by ration C in both the second and third periods, and likewise B by A and C by B. If A is the poorest ration and C the best, the milk yields for ration A may be increased by the beneficial carry-over effect of C, while those for ration C may be depressed by the carry-over effect of A. In these circumstances, the simple averages would *underestimate* the differences between the direct effects of the rations.

Thus when carry-over effects are present, the average milk yields under each ration must be adjusted in some way to avoid bias. These adjustments can clearly be made only if the design of the experiment enables us to estimate the sizes of the carry-over effects. This cannot be done with the design shown in figure 1, since each ration is always followed by the same ration. Thus, if this design is used for all six groups, no corrections for carry-over effects are possible, except perhaps by inspecting the daily yields, omitting from the results those parts of the experimental periods which appear to show carry-over effects. This method is at best somewhat arbitrary, and would be unsatisfactory if carry-over effects persisted through most of the succeeding periods.

However, by using both the cycles shown in figures 1 and 2, a direct evaluation of the carry-over effects is possible. This may be seen by examining the total milk yields for each of the six cycles, shown in table 5.

TABLE 5

Total milk yields for each of the six ration cycles
(Totals for 3 cows (18 weeks) in lbs.)

Period	Sets*											
	1		2		3		4		5		6	
I	A	4,383	B	5,370	C	5,720	A	4,427	B	4,675	C	5,236
II	B	4,057	C	4,980	A	4,208	C	4,535	A	3,637	B	4,420
III	C	3,834	A	3,264	B	3,571	B	3,833	C	3,787	A	2,956
Set totals		12,274		13,614		13,499		12,795		12,099		12,612
Grand total		76,893										
Ration totals:	A = Roughage A = 22,875			B = Limited grain B = 25,926			C = Full grain C = 28,092					

* To complete set 1, in which a cow was rejected, values were inserted for the missing cow by a method described below.

The results during the first period do not require adjustment, since all cows received the same preceding ration. In the second period, two sets of cows, 3 and 5, received roughage. Of these, set 3, having had full grain in the previous period, gave a total of 4208 lbs., while set 5, having had limited grain in the previous period, gave a total of 3637 lbs. The difference, 571 lbs., is an estimate of the difference between the carry-over effect

of full grain and that of limited grain. Similarly, a comparison of the carry-over effects of full grain and roughage is obtained from sets 1 and 6 in the second period, and a comparison between limited grain and roughage from sets 2 and 4. The same comparisons are repeated with the results in the third period. In all six comparisons, the carry-over effect was in the same direction as the direct effect (*i.e.*, full grain > limited grain > roughage), indicating strongly that real carry-over effects were present.

We may now consider how to obtain estimates of the direct effects of the rations, free from the disturbance due to carry-over effects. This may be done in many ways. For instance, an unbiased comparison of the direct effects of roughage and limited grain in each period is given by the mean milk yields of the following groups of cows:

Period	Roughage	Limited grain
I	Sets 1 and 4	Sets 2 and 5
II	Set 3	Set 6
III	Set 2	Set 4

Since sets 3 and 6 both had full grain in the first period, their yields in the second period, under roughage and limited grain respectively, are subject to the same carry-over effect, and similarly for sets 2 and 4 in the third period. An estimate of the difference between the two rations for the whole experiment could be obtained by taking some simple average of the differences in the individual periods. Such an estimate is, however, open to criticism on the grounds that only half of the available cows are used in the second and third periods. A further objection, probably more serious, is that the estimate would be partly a group comparison, since only the cows in sets 2 and 4 are common to both roughage and limited grain. The use of such an estimate would therefore defeat the purpose of the switch-over design, which is to avoid group comparisons.

To retain the full advantage of the switch-over design, the estimate of the difference between the effects of two rations must clearly be unaffected by differences in the yielding ability of the cows or by differences between the average yields for different periods. The most accurate estimates, subject to these restrictions, are given by a technique known as the method of least squares, which is frequently used for dealing with similar problems arising in field experiments. In this procedure, the observed milk yield for any cow in any period is expressed as a linear function of the effects of the cow's yielding ability, the period, the current and previous rations, and the experimental error. For example, consider a cow receiving in succession limited grain, full grain and roughage. Its milk yields y_1, y_2, y_3 in the three periods are expressed as follows:

$$y_1 = m + p_1 + d_b + e_1$$
$$y_2 = m + p_2 + d_c + r_b + e_2$$
$$y_3 = m + p_3 + d_a + r_c + e_3$$

where m = mean yield for the cow
p_1, p_2, p_3 = effects of the three periods
d_a, d_b, d_c = direct effects of the rations
r_b, r_c = residual effects of the rations given in the previous period
e_1, e_2, e_3 = experimental errors.

No parameter is required to represent residual effects in the first period. The constants p_1, p_2, p_3 remain unchanged for all three cows in the same group, since only the group averages of the differences between periods are eliminated from the experimental errors. After setting up equations of this type for every cow, the constants are estimated by minimizing the sum of squares of the experimental errors. The details of the mathematical analysis will not be given here. To calculate the adjusted yields given by the least squares solution, the following quantities (Table 6) are required, all being easily obtainable from table 5.

TABLE 6

Data required for calculating the adjusted yields for each ration

A	22,875	a	15,950	$s_2 + s_6$	26,226		
B	25,926	b	15,407	$s_3 + s_4$	26,294	T	76,893
C	28,092	c	15,725	$s_1 + s_5$	24,373	(2/3)T	51,262

Here A, B, C are simply the total yields under the three rations, as given at the foot of table 5. The quantities a, b, c are the total yields of all cows receiving these respective rations *in the previous period.*

e.g., a = 4057 + 3571 + 4535 + 3787 = 15,950, etc.

Also $s_2 + s_6$ = total of sets 2 and 6 = 13,614 + 12,612 = 26,226, etc.[1] Finally, we required two-thirds of the total of all yields in table 6 [$(\frac{2}{3})$ T = 51,262]. The adjusted mean yields per cow per period (total of six weeks) are then given by the following equations:

Roughage

$$72\bar{y}_a = 5A + (2a - b - c) + s_2 + s_6 - (\tfrac{2}{3})\,T$$
$$= 5 \times 22{,}875 + (2 \times 15{,}950 - 15{,}407 - 15{,}725) + 26{,}226 - 51{,}262$$
$$= 90{,}107$$

Hence $\bar{y}_a = 1251.5$

Limited grain

$72\bar{y}_b = 5B + (2b - a - c) + s_3 + s_4 - (\frac{2}{3})T = 103{,}801$ giving $\bar{y}_b = 1441.7$

Full grain

$72\bar{y}_c = 5C + (2c - a - b) + s_1 + s_5 - (\frac{2}{3})T = 113{,}664$ giving $\bar{y}_c = 1578.7$

The corresponding unadjusted mean yields per cow per period are the totals A, B and C, divided by 18 (the number of cows in each total).

The adjustments reduced the mean yield under roughage by 19 lbs., and increased the mean yield under full grain by about the same amount, these

[1] Sets 2 and 6 received roughage during period III; Sets 3 and 4 received limited grain, and Sets 1 and 5 full grain.

TABLE 7

Mean yields of fat corrected milk per cow per period of six weeks

Ration	Unadjusted			Adjusted for carry-over effects		
	Mean	Increase over roughage		Mean	Increase over roughage	
	lbs.	*lbs.*	*%*	*lbs.*	*lbs.*	*%*
A	1270.8	.....	.....	1251.5	.....	.....
B	1440.3	+169.5	+13.3	1441.7	+190.2	+15.2
C	1560.7	+289.9	+22.8	1578.7	+327.2	+26.1
		Standard error		± 21.4	± 30.3	± 2.4

effects being in the direction anticipated by the previous discussion. It is interesting to observe that the mean yield is practically unaltered for the cows receiving limited grain, for which the beneficial carry-over effect from full grain and the detrimental effect from roughage appeared to cancel. By failure to adjust for carry-over effects, the differences between the rations would have been underestimated by about 11 per cent.

To verify that the yields have been freed from carry-over effects, we may create artificially an additional carry-over effect of say, the full grain ration, by adding 1000 to the yields in table 5, wherever full grain was fed in the previous period. The yields affected are 3264 (set 2), 4208 (set 3), 3833 (set 4) and 4420 (set 6). With this change, the reader may verify that the adjusted mean yields become:

Roughage 1325.6 lbs., Limited grain 1515.8 lbs., Full grain 1652.7 lbs.

The effect of the artificial carry-over effect is simply to increase all three means by the same amount, 74.1 lbs., the differences between the ration means remaining exactly the same as before. With unadjusted means, on the other hand, the yields for roughage and limited grain would both be increased by 111.1 lbs., that for full grain being unchanged. The same property holds for the adjusted mean yields if a constant amount is added to all yields following any one of the three rations.

It may be of interest to examine the differences between the carry-over effects of the three rations. For a single cow (total of six weeks), these are given by the deviations of the following quantities (Table 8) from their mean.

TABLE 8

Estimates of the carry-over effects per cow (total for 6 weeks)

		Deviation (lbs.)
Roughage:	$(3a + A + s_2 + s_6)/24 = 96{,}951/24 = 4{,}040$	−58
Limited grain:	$(3b + B + s_3 + s_4)/24 = 98{,}441/24 = 4{,}102$	+ 4
Full grain:	$(3c + C + s_1 + s_5)/24 = 99{,}640/24 = 4{,}152$	+54

The difference in carry-over effect between roughage and full grain was 112 lbs., about one-third of the corresponding difference (327 lbs.) between the direct effects. The same ratio holds approximately for the other two differences between the rations. The carry-over effects seem strikingly large in relation to the direct effects; however, the figures given above agree well with the rough estimates which may be made from table 5. The six cows receiving limited grain during the second and third periods and full grain in the previous period gave a total yield of $4420 + 3833 = 8253$ lbs., as compared with 7628 lbs. for the six cows having limited grain preceded by roughage. The difference, 625 lbs., corresponds to 104 lbs. per cow, which checks closely with the estimate of 112 lbs. given above.

The adjustments required for the direct effects may be obtained from the above estimates for the carry-over effects. For example, of the eighteen yields from cows receiving roughage, six followed a previous ration of limited grain, six followed full grain, while none followed roughage. Thus the total yield of the eighteen cows should be reduced by six times the sum of the carry-over effects for limited grain and roughage, *i.e.*, the mean should be reduced by

$$(6/18)\ [54 + 4] = 58/3 = 19.3 \text{ lbs.};$$

in agreement with the reduction already given in table 7.

THE ANALYSIS OF VARIANCE WHEN CARRY-OVER EFFECTS ARE PRESENT

In analyzing the results for total nutrient consumption, we discarded results for the group containing the cow which was removed from the experiment. While this is the simplest procedure, it results in an appreciable loss of information. By a technique described by Yates (4) it is possible to include in the analysis the two healthy cows in group 3. The first step in this technique is to estimate results for the missing cow by the method of least squares. The completed data can then be analyzed by the usual procedure, with certain slight changes mentioned below.

The individual yields of fat corrected milk are shown in table 9, the values inserted for the missing cow being denoted by asterisks. In the analysis of variance, the total sum of squares and the sums of squares for groups, cows within groups, periods and the periods by groups interaction are found in the same way as for total nutrient consumption. Only the sums of squares between rations require special consideration. Of these, two degrees of freedom measure differences between the direct effects of the three rations, and two measure differences between the carry-over effects. However, the two sets of two degrees of freedom do not add to the correct total sum of squares for direct and carry-over effects, because the two effects are entangled by the nature of the design. This total sum of squares (4 degrees of freedom) may be obtained either as direct effects (ignoring carry-over effects) + carry-over effects, or as carry-over effects (ignoring direct effects) + direct effects.

TABLE 9

Individual yields of fat corrected milk units: lbs. per cow per period (total of six weeks)

Group 1								Group 2							
Period	Cow 1		Cow 2		Cow 3		Totals	Cow 4		Cow 5		Cow 6		Totals	
I	A	1376	B	2088	C	2238	5702	A	1863	B	1748	C	2012	5623	
II	B	1246	C	1864	A	1724	4834	C	1755	A	1353	B	1626	4734	
III	C	1151	A	1392	B	1272	3815	B	1462	C	1339	A	1010	3811	
Totals		3773		5344		5234	14351		5080		4440		4648	14168	

Group 3								Group 4							
Period	Cow 7		Cow 8		Cow 9		Totals	Cow 10		Cow 11		Cow 12		Totals	
I	A	1665*	B	1938	C	1855	5458	A	1384	B	1640	C	1677	4701	
II	B	1517*	C	1804	A	1298	4619	C	1535	A	1284	B	1497	4316	
III	C	1366*	A	969	B	1233	3568	B	1289	C	1370	A	1059	3718	
Totals		4548*		4711		4386	13645		4208		4294		4233	12735	

Group 5								Group 6							
Period	Cow 13		Cow 14		Cow 15		Totals	Cow 16		Cow 17		Cow 18		Totals	
I	A	1342	B	1344	C	1627	4313	A	1180	B	1287	C	1547	4014	
II	B	1294	C	1312	A	1186	3792	C	1245	A	1000	B	1297	3542	
III	C	1317	A	903	B	1066	3286	B	1082	C	1078	A	887	3047	
Totals		3953		3559		3879	11391		3507		3365		3731	10603	

A = Roughage, B = Limited grain, C = Full grain.
* Values inserted for cow which was rejected during the first period.

The sum of squares for direct effects (ignoring carry-over effects) is found in the same way as for total nutrient consumption, being:

$$(1/18)\ [(22{,}875)^2 + (25{,}926)^2 + (28{,}092)^2 - 1/3\ (76{,}893)^2] = 763{,}282.$$

The sum of squares for direct effects (adjusted for carry-over effects) is obtained from the adjusted ration totals given above (p. 944), as follows:

$$(1/360)\ [(90{,}107)^2 + (103{,}801)^2 + (113{,}664)^2 - 1/3\ (307{,}572)^2] = 777{,}534.$$

To calculate the two sums of squares for carry-over effects, we require the totals for the three rations, with and without adjustment for direct effects. The former have already been obtained (Table 8).

The adjusted sum of squares is:

$$(1/72)\ [(96{,}951)^2 + (98{,}441)^2 + (99{,}640)^2 - 1/3\ (295{,}032)^2] = 50{,}409.$$

The unadjusted totals are given by subtracting A, B and C, respectively from the corresponding adjusted totals. The sum of squares is:

$$(1/90)\ [(74{,}076)^2 + (72{,}515)^2 + (71{,}548)^2 - 1/3\ (218{,}139)^2)] = 36{,}158.$$

As a check, we may note that:

$$763{,}282 + 50{,}409 = 813{,}691 : 777{,}534 + 36{,}158 = 813{,}692;$$

this figure being the total sum of squares (4 degrees of freedom) for direct and residual effects. The error sum of squares is found by subtraction.

TABLE 10

Analysis of variance of fat corrected milk units: total yield per cow per period, in lbs.

	Degrees of freedom	Sum of squares	Mean squares
Total	50	5,124,267	
Periods	2	2,041,769	
Interaction of periods with groups	10	193,341	19,334
Groups	5	1,311,769	262,354
Cows within groups	11*	654,638	59,513
Rotations: Residual (ignoring direct)	2	36,158	
Rotations: Direct	2	777,534	388,767
Direct (ignoring residual)	2	763,282	
Residual	2	50,409	25,204
Error	18†	109,058	6,059

* One degree of freedom subtracted for missing cow.
† Two degrees of freedom subtracted for missing cow.

The complete analysis of variance is shown in table 10, both sets of 2 degrees of freedom for direct and carry-over effects being included. While only one set need be calculated to obtain the error sum of squares by subtraction, both are required if tests of significance of the direct and carry-over effects are desired. The mean squares for direct effects (388,767) and carry-over effects (25,204), are both significant, the 5 per cent F-value for 2 and 18 degrees of freedom being 3.55. The estimated standard error per cow (total of six weeks) is 77.84 lbs., or 5.5 per cent of the mean yield of 1424 lbs. The corresponding standard error for the mean of 18 cows would be $77.84/\sqrt{18}$. However, this must be increased to allow for the missing cow, and for the adjustments necessary to correct for carry-over effects. The effect of the missing cow is to decrease the effective replication from 18 to nearly 16.5, while the adjustments increase the standard error by the factor $\sqrt{1.25}$. Thus the standard error of the adjusted mean yields in table 7 is $77.84 \times \sqrt{1.25}/\sqrt{16.5} = 21.4$ lbs., or 1.5 per cent of the mean. The design attained a satisfactory degree of precision; an observed increase of 5 per cent would have been detected as statistically significant, while the actual differences between the ration means were all highly significant.

It has already been noticed (table 1) that the rates of fall in yield from period I to period III differed greatly for the different groups of cows. This fact is reflected in the analysis of variance, the mean square for the interaction of periods with groups being 19,334 as against 6,059 for the error mean square. If the six cycles had been assigned to the eighteen cows at random, with three cows to each cycle, the estimated error mean square would have been approximately $(24 \times 6{,}059 + 10 \times 19{,}334)/34 = 9{,}963$. The device of first dividing the cows into six groups of three on the basis of expected yielding ability therefore resulted in a marked increase in precision.

Similarly, the mean square between cows in the same group (59,513) is much larger than the error mean square. This indicates that any estimates based on group comparisons would have been subject to much higher experimental errors than those avoiding the use of group comparisons.

There is, of course, nothing to be gained by adjusting the means in cases where there are no carry-over effects. The important feature of the design is that it enables corrections to be made where these are considered necessary. An examination of the records, presented as in table 5 will usually be sufficient to decide the issue. In more doubtful cases, the direct and residual effects should be calculated as above, comparing the mean square for residual effects with the error mean square in the analysis of variance. If the former mean square is noticeably higher than the latter, it is safer to make the adjustments.

OTHER NUMBERS OF REPLICATIONS AND TREATMENTS

In the above design, the experimental unit consists of six cows, one receiving each cycle. To retain the full balance of the design, the number of cows used must be a multiple of six. In an experiment with 6k cows, the calculations are exactly as described above, except that all divisors should be multiplied by k/3. Thus if 24 cows had been used, the expressions $72\bar{y}_a$, $72\bar{y}_b$, $72\bar{y}_c$ for the adjusted totals would be replaced by $96\bar{y}_a$, $96\bar{y}_b$, $96\bar{y}_c$ respectively, the righthand sides of the equations remaining unchanged. The same rule applies to the divisors in the analysis of variance. Where there are no missing cows, the standard error of the adjusted means is $\sqrt{\frac{1.25s^2}{6k}}$ where s^2 is the error mean square in the analysis of variance.

Adjustments of this type cannot be made when only two rations are being compared in a switch-over design, since each ration must always precede the other. To obtain direct information on carry-over effects, say in a single reversal trial, it would be necessary to include cows receiving the sequences AA and BB, as well as cows receiving the switch-over sequences AB and BA.

With four treatments there are twenty-four possible cycles. However, the important features of the design can be obtained with only twelve cows, by the use of the following trio of 4×4 Latin squares:

Period	Cows				Cows				Cows			
	1	2	3	4	5	6	7	8	9	10	11	12
I	A	B	C	D	A	B	C	D	A	B	C	D
II	B	A	D	C	D	C	B	A	C	D	A	B
III	C	D	A	B	B	A	D	C	D	C	B	A
IV	D	C	B	A	C	D	A	B	B	A	D	C

FIG. 3. Change-over design for four rations.

Of the three A's in period II, one follows B, one follows C and one follows D. The same is true for any ration in any period after the first. The quantities required for estimating the direct and residual effects of ration A (with and without adjustment) are shown below.

TABLE 11

Calculation of the direct and residual effects in an experiment with four rations

Effect	Totals	Divisors	
		For means	For analysis of variance
Direct (unadjusted)	A	12	12
Direct (adjusted)	$11A + (3a - b - c - d) + s_4 + s_7 + s_{10} - \frac{1}{2}T$	120	1320
Residual (unadjusted)	$4a + s_4 + s_7 + s_{10}$	.....	132
Residual (adjusted)	$4a + A + s_4 + s_7 + s_{10}$	30	120

The symbols here have the same definitions as on pages 14 and 15. The sets s_4, s_7, s_{10} are those in which A is given during the fourth period, the same rule applying to rations B, C and D. The first column of divisors gives the numbers by which the corresponding totals must be divided to obtain estimates per cow per period. The second column shows the divisors required in the analysis of variance; in each case these apply to the sums of squares of deviations of the totals for the four rations from their mean. Since the divisors are for a single unit of 12 cows, they must be multiplied by k where k units are used in the experiment. The formula for the standard error of the adjusted means per cow per period is $\sqrt{\frac{1.1s^2}{12k}}$, where s^2 is the error mean square.

Since four is probably the maximum number of rations that could be given in a single lactation, no designs are presented for higher numbers of rations.

It should be understood that the designs described above apply particularly to short-time trials. Since it is difficult to keep a group of cows going for several lactations without some losses, such designs would require modification for use in long-time trials (of 3–4 years). Animal losses in experiments of this type destroy the balance of the design, thus making a thorough and efficient analysis of the results difficult.

SUMMARY

The design and statistical analysis of a short-time switch-over trial comparing three rations are discussed in this paper. The principal objects of the design are to secure accurate comparisons of the effects of the rations and unbiased estimates of the experimental errors. The design also makes it possible to estimate and adjust for carry-over effects, which have usually

been ignored in switch-over trials. The appropriate statistical analyses are illustrated by numerical examples of the case in which carry-over effects are negligible (nutrient consumption) and the case in which they are not negligible (milk production). A corresponding design for four rations is briefly described.

A detailed report of the results of this feeding trial will be given in a future paper.

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THE EFFECT OF PROCESSING ON THE NITROGEN DISTRIBUTION IN MILK

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Knowledge of the changes that take place in the proteins when milk is heated is obscure and most studies that pertain to this subject have been confined largely to the amount of coagulation that occurs at different temperatures. Considerable work has been done in this field but the results as a whole are difficult to compare because different temperature-time relationships and various analytical methods have been used to study the N distribution. Such studies also are complicated by the variable composition of milk which has a direct influence on the stability of the milk proteins when they are subjected to heat.

In most studies concerning the N distribution in milk macro-Kjeldahl procedures have been used which necessitate the filtration and transfer of bulky precipitates. Furthermore large amounts of reagents must be used to separate certain protein fractions and consequently large volumes of filtrate result which must be used in the subsequent analyses.

The macro procedures for determining the N fractions of milk are tedious and time consuming and limit the amount of experimental work that may be accomplished in a given time. Since previous experiments (1) showed that a semimicro procedure was as accurate as the macro methods for the determination of total N in milk it seemed probable that the separation and analysis of the protein fractions of this product could be made by semimicro methods.

This investigation was conducted in order to (a) make a preliminary study of the effect of processing on the N distribution in some of the more common milk products and (b) determine the routine applicability of a semimicro method recommended by Rowland (2) for separating the protein fractions of milk.

PROCEDURE

The milk samples used in this investigation were taken from the milk delivered to the University Creamery. Mixed herd milk was used for these experiments because it would tend to be of more uniform composition and this would introduce less error in the comparisons of the data in the various tables.

All samples were taken at the indicated stages of processing and analyzed, in duplicate, as rapidly as possible after their preparation. Those

Received for publication June 18, 1941.

not being analyzed were stored at 40° F. until needed, removed and brought to room temperature by immersion in a warm water bath.

No preservatives were added to the samples and none were stored long enough for acidity to develop as a result of bacterial action.

After warming the milk to room temperature it was weighed with Mojonnier weighing crosses and pipets and the pipets emptied into volumetric flasks of the capacity indicated in the following procedure recommended (2) for separating the protein fractions of milk. (If desired, samples may be weighed directly into the volumetric flasks.)

SEPARATION OF THE PROTEIN FRACTIONS OF MILK

I. *Total Nitrogen*

Weigh 5 gm. of milk into a 100-ml. flask. Dilute to the mark with water, mix, and pipet 20 ml. into a 300-ml. Kjeldahl digestion flask.

II. *Non-Casein N*

Weigh 10 gm. of milk into a 100-ml. flask, add 70–80 ml. of water at 40° C. and 1 ml. of 10 per cent acetic acid solution. Mix and after 10 minutes add 1 ml. of normal sodium acetate[1] and mix again. Cool and make to the mark with water. Filter through quantitative paper and pipet 20 ml. of the filtrate into a 300 ml. Kjeldahl flask.

The non-casein N determined this way is reduced to correct for the volume occupied in the 100 ml. flask by the precipitate of casein and fat (0.5 ml.). The correction factor (2) is non-casein N $\times$ 0.995. In all analyses this factor has been used regardless of variation in the fat and casein contents of the milk and neglecting this variation introduces extremely small errors in the non-casein and the casein N values.

III. *Non-Protein N*

Weigh 10 gm. of milk into a 50-ml. flask. Dilute to the mark with 15 per cent trichloroacetic acid solution and mix immediately. Filter and pipet 20 ml. of the filtrate into a 300-ml. Kjeldahl flask.

IV. *Proteose-Peptone plus Non-Protein N*

Weigh 10 gm. of milk into a 100-ml. flask. Heat for 20 minutes at 95° C. and precipitate the denatured albumin and globulin with the casein as in II (non-casein N). Filter, and pipet 20 ml. of the filtrate into a 300-ml. Kjeldahl digestion flask.

V. *Globulin N*

Pipet 20 ml. of the filtrate from II (non-casein N) into a 50-ml. beaker. Add brom thymol blue indicator and 0.1 N sodium hydroxide until color

[1] Mold growth in the sodium acetate may be prevented by adding a few drops of chloroform.

shows a pH range within 6.8 to 7.2. Saturate with magnesium sulphate—about 21 gms. Set beaker aside at room temperature for at least 4 hours. Filter (suction—5-cm. filter) and wash with saturated magnesium sulphate solution. Transfer precipitate and filter paper to a 300-ml. Kjeldahl flask.

A semimicro-Kjeldahl method (1) was used to determine the N in the various protein fractions. Mercuric oxide was used as the catalyst and boric acid as the ammonia receiving agent.

Five direct determinations of the nitrogen fractions of milk are made by these methods: I—Total N; II—Non-casein N; III—Non-protein N; IV—Proteose-Peptone plus Non-protein N; V—Globulin N.

Other nitrogen fractions are determined by difference: Casein N = I–II; Albumin plus globulin N = II–IV; Albumin N = II–(IV plus V); Proteose-Peptone N = IV–III.

ANALYTICAL RESULTS

The N distribution in milk and the various milk products is tabulated as per cent nitrogen and the nitrogen in the various fractions is calculated as percentage of the total nitrogen.

Nitrogen Distribution in Homogenized Milk. Homogenized milk was analyzed for nitrogen distribution to study the effect of homogenization on the milk proteins.

TABLE 1

Nitrogen distribution in 4 per cent milk homogenized at 2500 lbs. pressure

Control—pasteurized whole milk containing 4 per cent fat. Some of the same milk, after pasteurization, was cooled to 135° F., homogenized with single stage at 2500 lbs. pressure and cooled to 40° F.

	Past.† control (per cent N)	Per cent of total N	Homo. 2500 lbs. (per cent N)	Per cent of total N
Total N	0.5408		0.5362	
Non-casein N	0.1106	20.45	0.1090	20.33
Non-protein N	0.0301	5.58	0.0294	5.48
Globulin N	0.0187	3.46	0.0174	3.25
Casein N	0.4302	79.55	0.4272	79.67
Albumin N	0.0434	8.03	0.0495	9.23
Proteose N*	0.0184	3.40	0.0127	2.37

* In these data proteose N is meant to include proteose-peptone N.

† Pasteurization, where indicated, means that the product has been heated for thirty minutes at 145° F. and cooled to 40° F.

In tables 2 and 3 the data reported were obtained by the analysis of 4 per cent whole milk processed the same as the product in table 1 except the milk was homogenized at a higher pressure.

In another experiment the fat content of the whole milk was increased to 8 per cent and the sample processed the same as the product referred to in table 1 except the milk was homogenized at 4000 lbs. pressure. The results are given in table 4.

TABLE 2

Nitrogen distribution in 4 per cent milk homogenized at 3500 lbs. pressure

	Past. control (per cent N)	Per cent of total N	Homo. 3500 lbs. (per cent N)	Per cent of total N
Total N	0.5288		0.5216	
Non-casein N	0.1015	19.19	0.0915	17.54
Non-protein N	0.0248	4.69	0.0252	4.83
Globulin N	0.0193	3.65	0.0093	1.78
Casein N	0.4273	80.81	0.4301	82.46
Albumin N	0.0414	7.83	0.0453	8.68
Proteose N	0.0160	3.03	0.0117	2.24

TABLE 3

Nitrogen distribution in 4 per cent milk homogenized at 5000 lbs. pressure

	Raw (per cent N)	Clarified at 70° F. (per cent N)	Past. (per cent N)	Homo. 5000 lbs. (per cent N)
Total N	0.5477	0.5611	0.5562	0.5356
Non-casein N	0.1147	0.1203	0.1143	0.1076
Non-protein N	0.0303	0.0323	0.0287	0.0282
Globulin N	0.0161	0.0185	0.0162	0.0145
Casein N	0.4330	0.4408	0.4419	0.4280
Albumin N	0.0537	0.0545	0.0510	0.0473
Proteose N	0.0146	0.0150	0.0184	0.0176
Data above calculated as percentage of the total nitrogen				
Non-casein N	20.94	21.44	20.55	20.09
Non-protein N	5.53	5.76	5.16	5.27
Globulin N	2.94	3.30	2.91	2.71
Casein N	79.24	78.56	79.45	79.91
Albumin N	9.80	9.71	9.17	8.83
Proteose N	2.67	2.67	3.31	3.29

TABLE 4

Nitrogen distribution in 8 per cent milk homogenized at 4000 lbs. pressure

	Past. control (per cent N)	Per cent of total N	Homo. 4000 lbs. (per cent N)	Per cent of total N
Total N	0.5052		0.5111	
Non-casein N	0.1094	21.66	0.0985	19.27
Non-protein N	0.0300	5.94	0.0313	6.12
Globulin N	0.0200	3.96	0.0212	4.15
Casein N	0.3958	78.35	0.4126	80.73
Albumin N	0.0442	8.75	0.0354	6.93
Proteose N	0.0152	3.01	0.0106	2.07

The N distribution in homogenized skim milk (table 5) and homogenized 4 per cent raw milk (table 6) was studied to make comparisons with the previous tables (1–4). The skim milk was processed the same as the product referred to in table 1 and the raw milk was homogenized cold at 2500 lbs. pressure with single stage.

TABLE 5

Nitrogen distribution in skim milk homogenized at 3500 lbs. pressure

	Past. control (per cent N)	Per cent of total N	Homo. 3500 lbs. (per cent N)	Per cent of total N
Total N	0.5204	.....	0.5207	.....
Non-casein N	0.1099	21.12	0.1093	20.99
Non-protein N	0.0290	5.57	0.0295	5.67
Globulin N	0.0182	3.50	0.0152	2.92
Casein N	0.4105	78.88	0.4114	79.01
Albumin N	0.0436	8.38	0.0466	8.95
Proteose N	0.0191	3.67	0.0180	3.46

TABLE 6

Nitrogen distribution in raw milk homogenized at 2500 lbs. pressure

	Raw (per cent N)	Per cent of total N	Homo. 2500 lbs.	Per cent of total N
Total N	0.4861	.....	0.4848	.....
Non-casein N	0.1109	22.81	0.1111	22.92
Non-protein N	0.0289	5.95	0.0299	6.19
Globulin N	0.0178	3.66	0.0178	3.67
Casein N	0.3752	77.19	0.3737	77.08
Albumin N	0.0489	10.06	0.0504	10.40
Proteose N	0.0153	3.15	0.0130	2.68

Nitrogen Distribution in Evaporated Milk. Table 7 shows the N distribution in evaporated milk. The samples were taken during the various stages of the condensing process, as indicated in table 7, and analyzed for N distribution. The milk was condensed from 12.86 to 26.36 per cent total solids and 3.9 to 7.81 per cent fat. The condensed milk (D) after homogenization was canned and sterilized. No salts were added to the milk before sterilization. The raw, pasteurized and forewarmed milks were analyzed without any change while the condensed products were diluted with distilled water in order to bring the total solids concentration to 12.86 so that comparisons in N distribution could be made with the raw milk.

The data in table 7 show that significant changes have taken place in the protein fractions. Lower values are reported for the N fractions in the forewarmed sample but this is due to dilution of the milk with the steam condensate during the forewarming.

By the time the forewarming is completed all the albumin has been coagulated and this is determined (co-precipitated with casein at a pH of 4.6) with the casein N causing high results for this fraction. These changes are more significant in all samples of the condensed milk (C), (D) and (E).

It is interesting to compare the N distribution in the raw milk with that of the condensed milk (C). The casein N in (C) has increased 0.0753 per cent as a result of the heating while the non-casein N decreased 0.0749 per cent showing that the proteins (coagulated albumin and globulin) have

TABLE 7

Nitrogen distribution in evaporated milk

	Raw (per cent N)	Past. (per cent N)	Forewarmed 203° F. (per cent N)	*Condensed (C) (per cent N)	*Condensed (D) Homo. 2000 lbs. (per cent N)	*Condensed (E) Homo. 2000 lbs. Sterilized 240° F.–15″ (per cent N)
Total N	0.5333	0.5390	0.5026	0.5337	0.5380	0.5278
Non-casein N	0.1181	0.1128	0.0450	0.0432	0.0439	0.0593
Non-protein N	0.0297	0.0310	0.0282	0.0301	0.0309	0.0348
Globulin N	0.0139	0.0109	0.0119	0.0066	0.0075	0.0137
Casein N	0.4152	0.4262	0.4576	0.4905	0.4941	0.4685
Albumin N	0.0573	0.0530	0.0000	0.0000	0.0000	0.0000
Proteose N	0.0172	0.0179	0.0164	0.0158	0.0146	0.0187
			Data above calculated as percentage of total nitrogen			
Non-casein N	22.15	20.93	8.95	8.09	8.16	11.24
Non-protein N	5.57	5.75	5.62	5.64	5.74	6.59
Globulin N	2.61	2.02	2.37	1.24	1.39	2.60
Casein N	77.85	79.07	91.05	91.91	91.84	88.76
Albumin N	10.74	9.83	00.00	00.00	00.00	00.00
Proteose N	3.23	3.32	3.26	2.96	2.71	3.54

* Diluted with distilled water to bring the total solids concentration to 12.86 or the T. S. concentration of the raw milk.

been removed from the non-casein or soluble protein fraction. The total albumin and globulin N present in the raw milk amounts to 0.0712 per cent while the condensed milk (C) shows no albumin and 0.0066 per cent globulin N indicating that the increase in casein N accounts for slightly more than the amount of albumin and globulin coagulated. The albumin and globulin coagulated amount to 0.0646 per cent N and the difference between this value and the increase in casein N (0.0753) is only 0.0107 per cent N which is within the limits of experimental error.

A comparison of the casein N fraction of the condensed milk (C) with the final product (E) would indicate that some hydrolysis of this fraction has taken place.

Nitrogen Distribution in Condensed Skim Milk. Since evaporated milk showed variations in the N distribution it seemed advisable to study the effect of the condensing process on the protein fractions of skim milk.

Pasteurized skim milk was forewarmed to 150° F. and condensed from 9.20 to 31.37 per cent total solids. The condensed samples were diluted with distilled water before analysis to bring the total solids concentration back to that of the original skim milk. The N distribution of this product is shown in table 8.

TABLE 8

Nitrogen distribution in condensed skim milk

	Past. skim (per cent N)	Forewarmed 150° F. (per cent N)	Condensed (per cent N)
Total N	0.5367	0.5395	0.5453
Non-casein N	0.1118	0.1016	0.1086
Non-protein N	0.0293	0.0291	0.0305
Globulin N	0.0143	0.0150	0.0153
Casein N	0.4249	0.4379	0.4367
Albumin N	0.0464	0.0395	0.0396
Proteose N	0.0218	0.0180	0.0232
Data above calculated as percentage of total N			
Non-casein N	20.83	18.83	19.92
Non-protein N	5.46	5.39	5.59
Globulin N	2.66	2.78	2.81
Casein N	79.17	81.17	80.08
Albumin N	8.65	7.32	7.26
Proteose N	4.06	3.34	4.25

The changes in the protein fractions in table 8 are very small. Regardless of these minor changes there is evidence to show that the value for the casein N fraction has increased due to the co-precipitation of coagulated protein which appears to be albumin. The analysis of the data in table 7 supports this trend of thought.

Nitrogen Distribution in Cultured Milk. An analysis of cultured milk is shown in table 9. Pasteurized skim milk was repasteurized for 30 minutes

at 190° F., cooled to 70° F. and starter added (1 quart of starter to 25 gallons of milk). The milk was allowed to stand overnight and the following morning it was thoroughly mixed and a sample taken for analysis. The cultured milk was analyzed for nitrogen distribution to check the efficiency of the buffered solutions used in the determinations of non-casein N and proteose-peptone plus non-protein nitrogen. This seemed to be a practical method to determine the effect of developed acidity on the separation of these protein fractions. The acidity of the repasteurized milk was 0.14 and that of the cultured milk was 0.79 per cent.

TABLE 9

Nitrogen distribution in cultured skim milk

	Past. skim 145° F. (per cent N)	Past. skim 190° F. (per cent N)	Cultured (per cent N)
Total N	0.5203	0.5275	0.5233
Non-casein N	0.1155	0.0542	0.0694
Non-protein N	0.0317	0.0412	0.0479
Globulin N	0.0231	0.0167	0.0213
Casein N	0.4048	0.4733	0.4539
Albumin N	0.0424	0.0000	0.0000
Proteose N	0.0183	0.0156	0.0264
Data above calculated as percentage of total N			
Non-casein N	22.20	10.27	13.26
Non-protein N	6.09	7.81	9.15
Globulin N	4.44	3.17	4.07
Casein N	77.80	89.73	86.74
Albumin N	8.15	00.00	00.00
Proteose N	3.52	2.96	5.04

Repasteurization of the skim milk at 190° F. has coagulated all of the albumin and probably some globulin as indicated by the apparent increase in casein N in this sample. The decrease in the casein N in the cultured milk sample is attributed to a slight hydrolysis of this fraction. By comparing this sample with the enzyme treated milks (table 10) one will observe that the changes in N distribution are less significant but are of a similar nature.

The buffers used (sodium acetate and acetic acid) seem to function rather efficiently in separating the indicated protein fractions in the cultured milk regardless of the high acidity.

Several samples of skim milk which were sealed in tin cans, sterilized and cultured with a minimum amount of starter showed a N distribution comparable to the data in table 9.

Nitrogen Distribution in Enzyme Treated Milks. Four per cent raw milk was heated to 145° F. in glass stoppered bottles and then Enzylac, Trypsin and Steapsin added in a concentration of 1 part of enzyme to 25,000 parts of milk. The pasteurization process was completed and all samples analyzed for N distribution. The results are shown in table 10.

TABLE 10

Nitrogen distribution in enzyme treated milks

	Control Past. 145°–30″ (per cent N)	Steapsin at 145°–Past. (per cent N)	Trypsin at 145°–Past. (per cent N)	Enzylac at 145°–Past. (per cent N)
Total N	0.5194	0.5327	0.5286	0.5138
Non-casein N	0.1098	0.1365	0.1492	0.1326
Non-protein N	0.0335	0.0419	0.0531	0.0414
Casein N	0.4096	0.3962	0.3794	0.3812
Alb. and Glob. N*	0.0523	0.0521	0.0657	0.0483
Proteose N	0.0240	0.0425	0.0304	0.0429
Data above calculated as percentage of total N				
Non-casein N	21.14	25.62	28.23	25.81
Non-protein N	6.45	7.88	10.05	8.06
Casein N	78.86	74.38	71.77	74.19
Alb. and Glob. N	10.07	9.80	12.43	9.40
Proteose N	4.62	7.80	5.75	8.35

* Albumin and globulin determined together.

The enzyme treated milks show definite protein hydrolysis especially of the casein N fraction. This is more evident in the Trypsin and Enzylac treated milks. The non-casein, non-protein and proteose N fractions of the enzyme treated products have increased in percentage N. The data indicate that the cleavage products from the casein N fraction are determined with the other N fractions and especially with the non-casein N. The more significant increase in non-protein N in this data indicate that a greater degree of hydrolysis has taken place in the protein fractions of this product than in the evaporated milk—(E) table 7.

Average Nitrogen Distribution in Milk. Table 11 shows the average N distribution in five different samples of each of the following products: raw

TABLE 11

Average N distribution in processed milk

	Raw (per cent N)	Past. (per cent N)	Homo. (per cent N)	Skim (per cent N)
Total N	0.5306	0.5356	0.5228	0.5249
Non-casein N	0.1153	0.1106	0.1074	0.1104
Non-protein N	0.0299	0.0298	0.0293	0.0291
Globulin N	0.0176	0.0173	0.0203	0.0184
Casein N	0.4153	0.4249	0.4153	0.4145
Albumin N	0.0511	0.0447	0.0431	0.0410
Proteose N	0.0168	0.0187	0.0146	0.0218
Data above calculated as per cent of total N				
Non-casein N	21.73	20.65	20.54	21.03
Non-protein N	5.64	5.56	5.60	5.54
Globulin N	3.32	3.23	3.88	3.51
Casein N	78.27	79.33	79.44	78.97
Albumin N	9.63	8.35	8.24	7.83
Proteose N	3.17	3.49	2.79	4.15

bulk milk, pasteurized milk 4 per cent fat, 4 per cent milk pasteurized—cooled to 135° F. and homogenized at 2500 lbs. pressure with single stage, and pasteurized skim milk. All the processed products were manufactured from the raw bulk milk.

The data in table 11 show that the processing has produced no significant changes in N distribution.

DISCUSSION

The changes in the N distribution of the homogenized milk (tables 1, 2, 5, and 6) are not significant. There is some evidence in the data recorded in tables 3 and 4 to indicate that there may have been some disturbance of the protein fractions but the evidence is slight and these products have been subjected to pressures that are not used commercially.

The N distribution in evaporated milk (table 7) shows some interesting variations. The most significant changes have occurred as a result of the coagulation of the albumin and globulin—especially albumin. All globulin should be coagulated at 72° C. (3) but complete coagulation, according to the analysis, has not taken place. This is probably due to errors since this fraction contains such a small amount of N. For routine analysis it is more convenient and probably more accurate to determine the albumin and globulin together since the quantitative separation and determination of the small amount of globulin in milk is of questionable accuracy. In order to determine the amount of hydrolysis in the evaporated milk the casein N fraction in (E) is compared with the same fraction in the condensed (C) or the condensed and homogenized milk (D) because the casein N has increased due to coagulated albumin and globulin and this is the only fraction which shows any significant hydrolysis. Small but consistent increases in the amino N of heated milk (4, 5) indicate that some hydrolysis takes place during the heating process. The decrease in the casein N fraction in the evaporated milk (E) is beyond the limits of experimental error. The non-casein N has increased a significant amount, comparing (E) with (C) or (D), while the other N fractions have increased a small amount, so small as a matter of fact that the increase could be attributed to experimental error. However these increases in the N fractions of the evaporated milk with a decrease in the casein N fraction indicate that the proteins in the casein N fraction are in a preliminary stage of breakdown and these cleavage products are distributed and determined with the other N fractions, apparently largely the non-casein N fraction. This trend of thought seems to be supported by the N distribution in the enzyme treated milk (table 10) which shows definite hydrolysis of the milk proteins.

During this study curd tensions were determined on several of the milk products analyzed for N distribution. The curd tension of the evaporated milk (E, table 7) was zero for both the evaporated and the reconstituted

product. The raw milk, pasteurized milk and the homogenized milk (table 1) had curd tensions of 57, 53, and 12 grams respectively. The enzyme treated milk (table 10) which shows the most significant hydrolysis of any of the products had an average curd tension of about 26 grams. If the curd tension is a reliable measure of the consistency of the milk curd it seems reasonable to assume that the greatest alteration in the physical conditions of the milk proteins have taken place in the evaporated milk. There seems to be no direct relationship between N distribution and curd tension because the enzyme treated milks show the most significant hydrolysis and they had the highest curd tension of the three processed products.

One fact should be mentioned concerning the determination of casein N in milk products that have been subjected to heat. The present accepted analytical methods (6) for this determination are subject to error and the magnitude of this error will depend upon the temperature and the length of time the milk has been heated. As milk is heated at increasing temperatures the amount of albumin and globulin coagulated increases and these coagulated proteins are co-precipitated with the casein causing high results for this fraction. The data in table 7 show that the raw milk had a casein content of 0.4152 per cent N which may be considered the normal casein N value for all samples listed in this table. Using this figure (0.4152) as the normal casein N value one finds that a determination of the casein N in the pasteurized milk or in (C), (D) or (E) would result in errors of 0.0110, 0.0753, 0.0789 and 0.0533 per cent nitrogen respectively—if this fraction is to be called casein N. This fraction in all heated milk has been referred to in this report as casein N in order to be consistent and avoid confusion but the term is erroneous when milk is heated sufficiently to cause some

TABLE 12

Nitrogen distribution in sterilized skim milk

	Semimicro analysis	
	Past. skim (per cent N)	Sterilized (per cent N)
Total N	0.5333	0.5270
Non-casein N	0.1103	0.0719
Non-protein N	0.0300	0.0401
Globulin N	0.0197	0.0188
Casein N	0.4230	0.4551
Albumin N	0.0353	0.0000
Proteose N	0.0253	0.0319
Data above calculated as percentage of total N		
Non-casein N	20.68	13.64
Non-protein N	5.63	7.61
Globulin N	3.69	3.57
Casein N	79.32	86.36
Albumin N	6.62	00.00
Proteose N	4.74	6.05

coagulation of the albumin and globulin. It has been stated that the casein N fraction in (E, table 7) shows some hydrolysis but it is difficult to tell what proteins are actually hydrolyzed due to the co-precipitation of albumin and globulin with this N fraction.

The error introduced in the determination of casein N in heated milk may be demonstrated by an analysis of the data in tables 12 and 13. In this experiment pasteurized skim milk was sealed in tin cans and sterilized at 120° C. for 30 minutes. The skim milk and the sterilized skim milk were analyzed for N distribution by semimicro and the Official macro methods (6).

Casein, albumin and residual N[2] were determined by the Official methods (6) and compared with the same fractions determined by the semimicro methods. The comparison is shown in table 13.

TABLE 13

Comparison of the N distribution in skim milk analyzed by the official and semimicro methods

	Official method		Semimicro method	
	Past. skim (per cent N)	Sterilized (per cent N)	Past. skim (per cent N)	Sterilized (per cent N)
Casein N	0.4050	0.4484	0.4230	0.4551
Albumin N	0.0535	0.0287	0.0353	0.0000
Residual N*	0.0713	0.0791	0.0750	0.0908
(Calculated) Total N	0.5298	0.5562	0.5333	0.5459

* The determination of residual N is not an Official Method. It has been included with these methods for convenience and because the Official Kjeldahl-Gunning-Arnold Method was used to determine the N in this fraction and for all other macro determinations. The filtrate from the albumin N determination was used to determine residual N.

The total N (table 13) is obtained by adding the values for the different N fractions. In the semimicro analysis the values for residual N are obtained by adding the values reported in table 12 for proteose, non-protein and globulin N which would correspond to the residual N determined by the Official method. The residual N reported in the semimicro analysis may be calculated also by subtracting the albumin N from the non-casein N. The increases in the casein N fraction, due to coagulated albumin and globulin, may be observed in the sterilized milk. The casein N fraction determined by semimicro methods has a slightly higher N value than the same fraction determined by the Official methods. This is attributed to several factors which will be discussed later. The errors introduced in the determination of the N fractions of milk by both analytical methods may be observed by the variations in the calculated total N as compared to the actual value 0.5333 per cent N.

A Note Concerning the Separation of the Protein Fractions of Milk by Semimicro Methods. The theories concerning the separations of the protein

[2] See footnote to table 13.

fractions of milk by semimicro methods have been omitted because they have been comprehensively discussed by Rowland (7, 8).

The following procedure was used to check the semimicro separations of the protein fractions. Samples of reconstituted evaporated milk (some of same milk analyzed in table 7) were weighed into 150 ml. beakers and the milk proteins precipitated as in separations II, III and IV (Procedure). These precipitates were collected on quantitative filter paper and washed with the precipitating agents in the same concentration as they were used in the actual precipitation. The precipitates and paper were transferred to 500-ml. Kjeldahl digestion flasks and the N in these determined by the Official-Kjeldahl-Gunning-Arnold Method (6). The proteins represented by each of these three determinations were compared with the corresponding N fractions obtained by the semimicro N distribution analysis of the evaporated milk. Since only filtrates are used in the semimicro procedure it seemed desirable to analyze the protein precipitates from these separations, thus making it possible to actually check the semimicro separation of the protein fractions of the milk. The heated milk was used for this experiment in order to complicate the separations. The results are shown in table 14.

TABLE 14

Accuracy of protein separations by semimicro methods

Analysis by Semimicro-Kjeldahl		Analysis by Macro-Kjeldahl	
	Per cent N	Precipitate	Per cent N
Total N	0.5342		
Non-casein N	0.0551		
Non-protein N	0.0421		
Globulin N	0.0152	II	0.4843
Casein N	0.4792	III	0.5042
Albumin N	0.0000	IV	0.4844
Proteose N	0.0124		

Precipitate II should contain only casein N, Precipitate III should contain casein, albumin, globulin and proteose-peptone N, while Precipitate IV should contain casein, albumin and globulin N. The N values for these fractions should check with the sum of the corresponding N values reported in the semimicro analysis. The comparisons are as follows:

	Macro-Kjeldahl Precipitates (per cent N)	Semimicro-Kjeldahl Filtrates (per cent N)	Difference (per cent N)
Casein	0.4843 (Ppt. II)	0.4792	0.0051
Casein Albumin Globulin Proteose	0.5042 (Ppt. III)	0.5068	0.0026
Casein Albumin Globulin	0.4844 (Ppt. IV)	0.4944	0.0100

The checks secured in this separation are satisfactory.

Another experiment of similar nature was conducted in order to compare all results with the present accepted Official Methods (6) for determining the proteins in milk. Pasteurized 4 per cent milk was used for this experiment. Table 15 shows the N distribution in this milk as determined by the Official Methods (in triplicate) and the semimicro methods (in duplicate).

TABLE 15

N distribution in pasteurized milk by official and semimicro methods. Results reported as per cent N

Official methods					
Total N	Official casein N	Tent. casein N	Albumin N	Residual N	
0.5108	0.4008	0.4110	0.0529	0.0626	
0.5057	0.3958	0.4021	0.0527	0.0613	
0.5131	0.3804	0.4083	0.0518	0.0610	
0.5099	0.3923	0.4071	0.0525	0.0616	Ave.

Semimicro methods			
Total N	0.5116	0.5184	0.5150
Non-casein N	0.1073	0.1080	0.1077
Non-protein N	0.0309	0.0309	0.0309
Proteose-peptone plus non-protein N	0.0472	0.0477	0.0475
Globulin N	0.0134	0.0155	0.0145
Casein N	0.4043	0.4104	0.4074
Albumin plus Globulin N	0.0601	0.0603	0.0602
Albumin N	0.0467	0.0448	0.0458
Proteose-peptone N	0.0163	0.0168	0.0166
Residual N (Calculated)	0.0620	0.0619	0.0620

Casein was determined by the Official and Tentative Methods in order to compare these determinations with the semimicro results for the same fraction. The Tentative Official Method is similar to the semimicro procedure for the determination of this protein and the data indicate that the results secured by each method check very closely. Both of these methods give more consistent checks for casein N than the Official method.

The Tentative Method is not adaptable to routine analyses because unnecessary steps have been introduced into the method which make it too inconvenient for general use. The simplified procedure indicated in separation II (Procedure) will give the same results, by either macro or semimicro methods, as the Tentative method with much less inconvenience to the analyst. Both Rowland (7) and Moir (9) have claimed that the Official Method for casein gives low results because the filtrates from this precipitation have a pH lower than the iso-electric point of casein. The filtrates from the casein precipitations by all three methods were checked and the pH found to be 3.3 for the Official and 4.6 for the Tentative Official and semimicro methods. The last two methods give higher values for casein N than

the Official Method (table 15) and this is attributed to the proper pH of the filtrate (pH 4.6) and the elimination of washing and transferring casein precipitates.

Samples of the same 4 per cent milk used for the previous experiment (table 15) were weighted into 150 ml. beakers and the proteins precipitated as in II, III, and IV (Procedure), or the same procedure was followed as was used to obtain the data shown in table 14. The analysis of the protein precipitates is shown in table 16.

TABLE 16

Accuracy of semimicro protein separations in 4 per cent milk. Results in per cent N

	Ppt. II	Ppt. III	Ppt. IV
	0.3908	0.4758	0.4543
	0.3831	0.4838	0.4531
	0.3908	0.4619	0.4549
Ave.	0.3882	0.4738	0.4541

The average N values for these protein fractions are compared with the corresponding average N values in the semimicro analysis reported in table 15. The results are as follows:

	Macro-Kjeldahl Ppts.	Semimicro-Kjeldahl filtrates	Difference
Casein	0.3882 (Ppt. II)	0.4074	0.0192
Casein Albumin Globulin Proteose	0.4738 (Ppt. III)	0.4843	0.0105
Casein Albumin Globulin	0.4541 (Ppt. IV)	0.4677	0.0136

The results are very satisfactory when one considers the difficulties involved in an analytical comparison of this nature. These results and those reported in table 14 indicate that the semimicro methods are satisfactory for separating the protein fractions of milk.

Five individual determinations were made on the same sample of pasteurized 4 per cent milk in order to determine the reliability of individual determinations by the semimicro methods. The results are shown in table 17.

Duplicate determinations on the same N fractions by semimicro methods usually give more satisfactory checks than macro methods. The semimicro methods for determining the protein fractions of milk are not above criticism but they are sensitive, well adapted to routine analyses and reliable as indicated by their comparison with the present accepted methods.

TABLE 17

Accuracy of individual determinations by semimicro methods. Results in per cent N

	Mean	Mean deviation
Total N	0.5187	0.0030
Non-casein N	0.1087	0.0009
Non-protein N	0.0298	0.0009
Proteose-peptone plus non-protein N	0.0493	0.0015
Globulin N	0.0104	0.0032
Casein N	0.4100	0.0026
Albumin and Globulin	0.0598	0.0012
Albumin	0.0488	0.0025
Proteose-peptone	0.0195	0.0024

SUMMARY

1. Semimicro methods have been described and used to study the N distribution in milk. These methods are efficient, well adapted to routine analyses and results compare very favorably with those obtained by the Official Methods.

2. The homogenization of milk at normal and abnormal pressures produced no significant changes in the N distribution.

3. The coagulation of albumin and globulin was the most significant change that occurred in the N distribution of evaporated milk. There is evidence to indicate that some hydrolysis of the proteins takes place in this milk as a result of the processing.

4. Condensing skim milk produced only minor changes in the N distribution of the product.

5. The addition of Steapsin, Trypsin and Enzylac to milk at a temperature of 145° F. for 30 minutes produced definite hydrolysis of the milk proteins.

6. Pasteurization of milk at 145° F. for 30 minutes produced no significant changes in N distribution.

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A STUDY IN CHEESE RIPENING. THE INFLUENCE OF AUTOLYZED CELLS OF *STREPTOCOCCUS CREMORIS* AND *STREPTOCOCCUS LACTIS* ON THE DEVELOPMENT OF *LACTOBACILLUS CASEI*

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There is a gradual change in the bacterial flora of cheese during the process of ripening. In the hard cheeses, which have not been heated to a high temperature, *Streptococcus lactis* and *Streptococcus cremoris* are predominant at first, later being replaced by *Lactobacillus casei* (Orla-Jensen) Holland (*Bacillus casei alpha* v. Freudenreich and Thöni, *Streptobacterium casei* Orla-Jensen), a bacterium which is of importance for the cheese ripening process on account of its proteolytic activity. Many strains of this species show active break-down of casein when inoculated into milk containing calcium carbonate (Orla-Jensen 1919 (1)). No satisfactory explanation for the general occurrence of *L. casei* has so far been given.[1] In the present work a factor for the development of *L. casei* has been studied, namely the influence of the cell content of *Str. lactis* and *Str. cremoris*.

EXPERIMENTAL

In the course of the study the following strains were used:

From the collection of Dr. S. Orla-Jensen, Copenhagen: *Streptococcus cremoris* No. 37, *Str. lactis* No. 7, *Str. lactis* No. 17, *Str. lactis* No. 22, *Betacoccus cremoris* No. 7, *Streptobacterium casei* No. 7, *Streptobacterium casei* No. 11 and *Thermobacterium helveticum* No. 12.

From the American Type Culture Collection, Washington, D. C.: *Lactobacillus casei* No. 334, *Lactobacillus casei* No. 393 = *Streptobacterium casei*, Orla-Jensen No. 7.

From the collection of Dr. J. M. Sherman, Cornell University, Ithaca, N. Y.: *Streptococcus cremoris* No. C37V.

The streptococci were grown in 4 liters of a peptic casein digest medium, which was made perfectly clear with albumin of egg, $\frac{1}{4}$ per cent being used.

Casein digest adjusted to 0.5 per cent nitrogen	1000 ml.
K_2HPO_4	2 g.
$MgSO_4 \cdot 7H_2O$	1 g.
Glucose	5 g.

adjusted to pH 6.8

Received for publication June 23, 1941.

[1] This organism should not be confused with *Lactobacillus helveticus* (Orla-Jensen) Bergey *et al.* (*Bacillus casei epsilon* v. Freudenreich and Thöni, *Thermobacterium helveticum* Orla-Jensen) which is of the greatest significance for the ripening of Swiss cheese, but, unlike *L. casei*, it is not generally found in all kinds of cheeses.

The cultures were incubated at 20–23° C. until a heavy growth resulted, usually for 2–6 days. The cultures were centrifuged, the supernatant fluid poured off, and the cells were suspended in one liter of physiological saline. The process was repeated, and finally the bacteria were suspended in a small volume of saline, about 40 ml. From then on the bacterial preparations were handled in various ways.

A. *Autolysis of Bacteria.* This was carried out by the following procedure. To the 40 ml. of suspension were added 1.7 g. NaCl and a little toluene, the mixture was well shaken and placed at 49° C. for 5 weeks. The acid formed during the process was neutralized by adding 0.2 N NaOH to a slightly alkaline reaction, the indicator, thymol blue, being used. The suspensions were examined under the microscope, and it was noticed that during this long exposure to 49° C., considerable break-down of the cells occurred, while, *e.g.*, 48 hours treatment seemed quite insufficient to change the appearance of the cells. After evaporation of the toluene on a water bath, the nitrogen content was ascertained and finally the preparation was diluted to a definite nitrogen content and sterilized by autoclaving in a Freudenreich flask.

B. *Disintegration of Bacteria by the Sonic Oscillator* (Chambers and Flosdorf 1936 (2)). The suspension of bacteria in physiological saline, brought to neutrality, and with a little toluene added was subjected to sonic vibration for a period of 10–30 minutes. The toluene was evaporated, nitrogen determined, and the preparation was diluted to a suitable nitrogen content and sterilized by autoclaving.

C. *No special treatment of bacteria other than sterilization.* Sterilization was usually carried out by 15 minutes exposure in the autoclave at 15 lbs. pressure.

In addition to skim milk and casein digest a whey medium was used in one instance, prepared according to a formula of Mr. M. Rogosa, Bureau of Dairy Industry, U. S. Department of Agriculture. Skim milk was heated to 37° C. and sufficient rennet added to form a firm coagulum in about 30 minutes. The curd was cut in rather big pieces, disturbing it as little as possible. The whey was heated to 80° C., cooled and kept in the ice box over night, then centrifuged; finally the supernatant liquid was passed through a Seitz filter EK. The resulting medium was filtered once more through a Seitz filter for sterilization and pipetted into sterile tubes. They were incubated at 30° C. for five days to detect possible contamination.

EXPERIMENT NO. 1

The influence of autolyzed cells of *Streptococcus lactis* (strain 22) on the development of *L. casei* (strains 7 and 11), *Str. lactis* (strain 22), and *Betacoccus cremoris* (strain 7) was studied. Skim milk to which varying amounts of extracts containing 0.5 per cent nitrogen had been added was

tubed in 10 ml. lots, and sterilized in the autoclave. The tubes were inoculated and incubated for 16 days, the *L. casei* strains at 30° C., *Str. lactis* and *Betacoccus cremoris* at 23° C. At the end of this period the cultures were titrated with NaOH and the results expressed in per cent lactic acid formed.

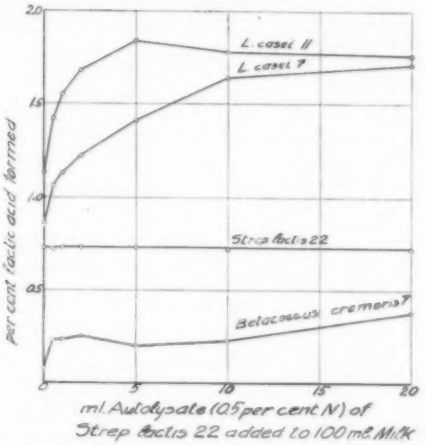
L. casei 11
L. casei 7
Strep lactis 22
Betacoccus cremoris 7
per cent lactic acid formed
ml. Autolysate (0.5 per cent N) of
Strep lactis 22 added to 100 ml. Milk

FIG. 1. Influence of autolyzed cells of *Str. lactis* 22 on acid formation by *L. casei* 7 and 11, *Str. lactis* 22, and *Betacoccus cremoris* 7.

The data in figure 1 show a considerable increase in the endpoint of fermentation for the *L. casei* strains and for *Betacoccus cremoris* 7; but *Str. lactis* 22 was not influenced by extracts of its own cells.

The activity was not limited to extracts of this strain as the following results show.

EXPERIMENT NO. 2

The experimental procedure was in the main the same as in No. 1 with the exception that the media in which the various *Strs.* were grown contained 1 per cent glucose and that the preparations of autolyzed cells were made up to 1 per cent nitrogen. Autolysates of *Str. lactis* (strains 7 and 17) and of *Str. cremoris* (strain 37) were added to milk as in the previous experiment. The organisms grown in the extracts were *L. casei* (strains 7 and 11) at 30° C. and *L. helveticus* (strain 12) at 37° C. *L. casei* was encouraged, as will be noted from figure 2, while *L. helveticus* seems not to be influenced favorably, but rather inhibited.

EXPERIMENT NO. 3

A suspension of *Str. cremoris* (C37V) was prepared and divided into two parts. One was left without special treatment and the other was shaken by sonic vibration for 10 minutes, the object being to ascertain whether the

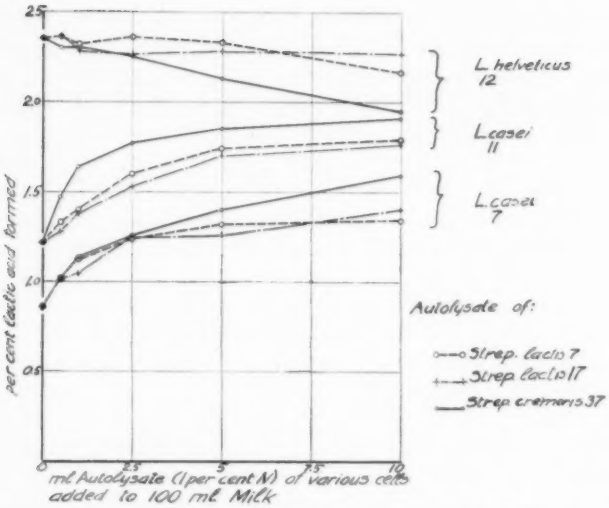
L. helveticus 12
L. casei 11
L. casei 7
Autolysate of:
Strep. lactis 7
Strep. lactis 17
Strep. cremoris 37
per cent lactic acid formed
ml Autolysate (1 per cent N) of various cells added to 100 ml. Milk

FIG. 2. Influence of autolyzed cells of *Str. lactis* 7 and 17 and *Str. cremoris* 37 on *L. helveticus* 12, *L. casei* 7 and 11.

breaking-up of the cells actually has any significance or whether an untreated plain cell suspension would not be just as active. One ml. of different dilutions of the suspensions was added to 9 ml. milk or whey, bringing the final volume to 10 ml. The resulting culture media tubes were inoculated and then incubated at 30° C.

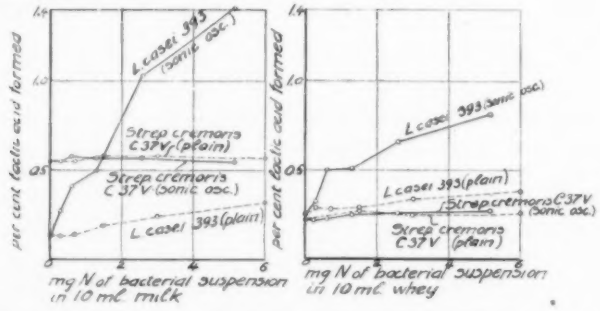
L casei 393 (sonic osc.)
Strep cremoris C37V (plain)
Strep cremoris C37V (sonic osc.)
L casei 393 (plain)
per cent lactic acid formed
mg N of bacterial suspension in 10 ml milk
L casei 393 (sonic osc.)
L casei 393 (plain)
Strep cremoris C37V (sonic osc.)
Strep cremoris C37V (plain)
mg N of bacterial suspension in 10 ml whey

FIG. 3. Influence of cell suspensions of *Str. cremoris* C37V on acid formation of *L. casei* 393 and *Str. cremoris* C37V in milk and whey.

Str. cremoris extracts have no influence on the development of a culture of the same strain; but the *L. casei* is very favorably acted upon, and the disintegrated cells of *Str. cremoris* are far more potent than the whole cells (see fig. 3).

TABLE 1

Endpoint of fermentation produced in milk to which cells and filtrates from cells of Streptococcus cremoris were added

Composition of media	*L. casei* 334		*L. casei* 393	
	Per cent lactic acid formed	Days to curdle	Per cent lactic acid formed	Days to curdle
8.1 ml. skim milk + 0.9 ml. physiol. saline	1.38	3	0.19	Did not
8.1 ml. skim milk + 0.9 ml. suspension, sonic vibrated (A)	1.49	3	0.65	3
8.1 ml. skim milk + 0.9 ml. plain suspension (B)	1.43	3	0.28	Did not
8.1 ml. skim milk + 0.9 ml. Seitz filtrate of A	1.52	3	0.65	3
8.1 ml. skim milk + 0.9 ml. Seitz filtrate of B	1.40	3	0.25	Did not

A = sonic vibrated suspension, 3.19 mg. N/ml.
B = plain vibrated suspension, 3.17 mg. N/ml.

TABLE 2

Endpoint of fermentation and growth produced in casein digest to which filtrates from cells of Streptococcus cremoris were added

Composition of media	*Streptococcus cremoris* C37V			*Lactobacillus casei* 334			*Lactobacillus casei* 393		
	Per cent lactic acid formed	Turbidity		Per cent lactic acid formed	Turbidity		Per cent lactic acid formed	Turbidity	
		E	$\frac{E}{E_{control}}$		E	$\frac{E}{E_{control}}$		E	$\frac{E}{E_{control}}$
Control. 4 ml. casein digest, double strength, with 80 mg. glucose + 4 ml. saline	0.38	0.40	1.0	0.69	0.26	1.0	0.31	0.20	1.0
4 ml. casein digest, double strength, with 80 mg. glucose + 1.2 ml. filtrate from A + 2.8 ml. saline	0.52	0.48	1.2	.....	.....	.....	0.56	0.51	2.5
4 ml. casein digest, double strength, with 80 mg. glucose + 2.4 ml. filtrate from A + 1.6 ml. saline	0.53	0.49	1.2	0.91	0.71	2.7	0.80	0.61	3.0
4 ml. casein digest, double strength, with 80 mg. glucose + 2.4 ml. filtrate from B + 1.6 ml. saline	0.48	0.44	1.1	0.92	0.61	2.3	0.44	0.33	1.65

A = sonic vibrated suspension.
B = plain suspension.

$E = \text{Extinction} = \log_{10} \frac{100}{\text{o/o transmission}}$

EXPERIMENT NO. 4

The experiments so far described were concerned with the endpoint of fermentation; but the crop of bacteria produced cannot be predicted from such data. Bacterial counts are difficult to evaluate in case of chain-forming rods, since an unbroken chain or a very long rod may produce one colony only as does a single short rod, when the plate method is used. Turbidity measurements were used in the present work; they seem to give satisfactory results, provided the apparatus is operated with a range of about 60–25 per cent transmission or an extinction of 0.22–0.60, and provided a sufficient number of control tubes are being run simultaneously with the inoculated tubes. The apparatus used was an Aminco Photometer, Type F, equipped with filter 58. Obviously a clear medium must be used; thus milk had to be abandoned and a casein digest containing one per cent glucose was used instead. The bacterial extract was prepared by shaking a suspension of cells for 30 minutes on the sonic oscillator, strain C37V of *Str. cremoris* being used. Besides studying the growth as determined by increase in turbidity the object of the present experiment was to determine whether the active principle would pass a filter. After inoculation the cultures were incubated at 30° C. for 30 days. The turbidity was always measured in relation to the corresponding noninoculated medium, but containing the various additions. Simultaneously another experiment using skim milk as a basic medium was run for the sake of comparison.

DISCUSSION

There is no proportionality between the amount of growth and the endpoint of fermentation. Yet it may be stated that for each single strain an increase in endpoint always corresponds to an increase in growth through the range investigated.

Figures 1 and 2 indicate definitely an increased development of all *L. casei* strains and of *Betacoccus cremoris*. The latter species is found in starters for butter-making together with *Str. lactis* and *Str. cremoris*. Although it develops scantily in pure culture in skim milk, it is found in starters, which have been transferred for years. The favorable influence of the cell content of the streptococci on the development of *Betacoccus cremoris* may be a significant factor. *L. helveticus* was not encouraged in the experiments here described; but it must be remembered that skim milk is already a very good culture medium for *L. helveticus* and that a different result may have been obtained if another basic medium had been used for the study. The decrease in endpoint with very large additions of extracts was undoubtedly caused by the fact that this organism is very sensitive to sodium chloride. Thus a somewhat distorted picture resulted when large amounts of autolyzed cells were added. (See figs. 1 and 2.) In the most striking case the final medium with 10 ml. extract of *Str. cremoris* 37 added per 100

ml. milk, contained 0.49 per cent sodium chloride. The two other extracts, of *Str. lactis* 7 and 17, had a smaller sodium chloride content per one per cent nitrogen, because the crops of these species were larger than those of *Str. cremoris* 37.

Figure 3 shows that even the suspension of untreated cells of *Str. cremoris* C37V were somewhat active; however the sonic vibrated cell suspension was very much more active towards *L. casei*. Two weeks intervened between the preparation of the sterile cell suspension and the starting of experiments No. 3 and 4, and some of the cell content may have passed into the surrounding medium during the storage. The data presented show the importance of using rigorous methods for liberating the cell content, at least when streptococci are studied.

The active principle seems to be filtrable as indicated by tables 1 and 2. The much greater activity of sonic vibrated suspensions as compared with control suspensions has also been demonstrated in this experiment. The strain *L. casei* 334 is less sensitive to additions than the other *L. casei* strains when acid formed is used as an indicator. The data in table 2 show that also with a casein digest medium stimulation of the *L. casei* strains measured either by turbidity or by acid formation can be demonstrated. Under the conditions of these experiments the growth was increased 2.7 times for *L. casei* 334 and 3 times for *L. casei* 393.

In this work the nitrogen content of the various extracts was determined with the object of roughly standardizing the strength of the active preparations. However the activity varied considerably and the nitrogen content is only a rough guide to the potency of the extracts. The endpoint of fermentation may, of course, vary a little with different lots of milk. Although this difference is usually insignificant the same lot of milk was always used throughout each single series of experiment.

CONCLUSIONS

1. Extracts of *Streptococcus cremoris* and *Streptococcus lactis* stimulate the development of *Lactobacillus casei* and *Betacoccus cremoris* (*Streptococcus citrovorus*). Both the endpoint of fermentation and the final bacterial crop are increased. However, no or only faint stimulation is observed when *Stretococcus lactis* and *Streptococcus cremoris* are grown in media enriched by their own cell content.

2. Preparations of autolyzed cells or sonic vibrated cells are much more potent than non-treated control suspensions of killed cells.

3. The active principle is filtrable through a Seitz filter EK.

4. The stimulation of *Lactobacillus casei* by *Streptococcus cremoris* and *Streptococcus lactis* may play an important role in the ripening of cheese, where these species are found at first, then die and disintegrate and are followed by *L. casei*. Furthermore the phenomenon may be of importance in

butter and cheese cultures where the two streptococci are found mixed with aromabacteria.

5. The sonic oscillator is a valuable tool for the study of associated growth of microorganisms.

ACKNOWLEDGMENT

The author is very much indebted to Drs. L. A. Rogers and R. P. Titsler, Dairy Research Laboratories, Bureau of Dairy Industry, Department of Agriculture for advice, suggestions, and laboratory facilities; and to Dr. S. Orla-Jensen, Royal Technical College, Copenhagen, and to Dr. J. M. Sherman, Cornell University, Ithaca, N. Y., for kindly placing their cultures at his disposal.

The author wishes also to express his appreciation to Dr. J. C. Hoogerheide of the Biochemical Research Foundation, Newark, Delaware, who kindly prepared the oscillated suspension.

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THE EFFECT OF VITAMIN A AND CERTAIN MEMBERS OF THE B-COMPLEX UPON CALF SCOURS[1]

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The causal factor or factors involved in "scours" in the early life of the calf have been largely considered to be bacterial in nature. There is little doubt that infectious agents are seriously involved and particularly so in the case of "white scours." That nutrition or the nutritive state of the calf is likewise of some importance in the course of such diseases has been taken for granted but little questioned. To our knowledge no specific nutritional researches have been made which would definitely indicate the role of nutrition as it relates to scours. Krauss and coworkers (4) have shown that the amount of carotene and vitamin A in the liver of the new-born calf is extremely low. It has long been known that colostrum milk is rich in vitamin A. Further, the function of vitamin A has always been associated with disease resistance.

In view of these facts and the rather common appearance of diarrhea among calves of certain herds under our observation, a field study was made to determine the effect of vitamin A upon the control of scours in the new-born calf.

Several herds which had been under observation for reproductive troubles first caused us to turn our attention to their calf crop. Generally speaking the cows in these troublesome herds which were causing concern were low in vitamin C and responded to both vitamin C and A treatment (8). Calves from these herds had shown the following history: The calves were apparently normal at birth. They were able to nurse but usually went "off feed" between the second and seventh days with early evidence of an effusive watery diarrhea, or scours. Frequently the feces bordered on the "white" type of scours and not infrequently there was some evidence of hemorrhage. Often there was excessive lacrimation although this was not a constant symptom. The course of the disease usually went into pneumonia in its final stages and death from the latter cause. Before treatment was instituted the disease proved to be fatal in 50 to 75 per cent of the cases and those which withstood the attack were greatly retarded in their development. Two herds, one a Holstein and the other a Jersey, were available for study together with a few isolated cases. In both herds the amount of milk fed was rigidly reduced with the onset of the diarrhea. In the case of the Jersey

Received for publication June 24, 1941.

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station. These studies were supported in part by a grant from the Wisconsin Alumni Research Foundation.

calves they were given regularly only 3 lbs. and the Holstein calves about 5 lbs. milk each per day. The owner of the Holstein herd had appealed to the State Sanitation Laboratory for aid. Upon their recommendation the pregnant cows were injected with a bacterin preparation before the calves were dropped and the new-born calves were immediately treated with the serum at birth. This routine had been in practice without favorable results for some two months prior to our vitamin A and C analyses and subsequent vitamin therapy. Carotene and vitamin A analyses were made with the Evelyn colorimeter using Kimble's method (3). The constant for the conversion of the L values into gamma of vitamin A was obtained from measurements with pure vitamin A.[2] Vitamin C analyses were made according to the Mindlin and Butler method (6).

Shark liver oil[3] containing 15,000 I.U. of vitamin A per gram was used as the source of vitamin A. It was fed in 5 cc. gelatin capsules at the rate of 10 cc. per week. In most cases the calves were fed for 2 or 3 weeks depending upon their condition.

RESULTS

The Holstein calves were treated with shark liver oil. In general there was an immediate response as shown by the improvement in appetite and general appearance of the calves. Diarrhea was only partially controlled. There was some temporary improvement in all the treated calves. However, only about half of the calves recovered sufficiently to show no effects of the disease. Those which did not recover were subsequently treated with certain members of the B complex as described below.

The second herd to be treated was a Jersey herd where the mortality was extremely high. Calf scour remedies were tried without success. Since we had not attained 100 per cent response in the Holstein calves to vitamin A administration it was decided to consider the administration of other vitamins as well. Studies on the pathology of nutritional diseases had shown in our laboratory (9) that (1) a nicotinic acid deficiency caused diarrhea in the dog, (2) that a pantothenic acid deficiency caused marked congestion of the entire intestinal tract and diarrhea in both the rat and dog, often accompanied by evidence of hemorrhage, and (3) that vitamin A deficiency likewise caused diarrhea. On the basis of these studies with other species and at the suggestion of Dr. D. F. Green of Merck and Co., certain members of the vitamin B complex[3] were obtained and fed. From analyses for the various members of the vitamin B complex in milk it was estimated from the known requirement of other species that the calf (100 lbs. live weight) would require approximately 10 lbs. of milk daily to supply his vitamin B com-

[2] Determined by Dr. F. W. Quackenbush and H. L. Gottlieb.

[3] We are indebted to Bioproducts Inc., Astoria, Oregon, for the generous supply of shark liver oil and to Merck and Co., Rahway, New Jersey, for their generous supply of the crystalline vitamin B complex used in these experiments.

plex requirements. The first experiments on 6 Jersey calves indicated that nicotinic and pantothenic acids together with shark liver oil completely controlled the scours in these calves. Since the calves were on a reduced milk intake and the management desired to continue them thus, other members of the B complex were added. Therefore the results with these calves were obtained on nicotinic and pantothenic acid for the first two weeks and the other B vitamins added for an additional 2 weeks. These vitamins were given in weighed quantities in capsules once per week at the rate of:

Vitamin B_1	1 mg./day
Riboflavin	1 " "
Pantothenic acid	5 " "
Choline	5 " "
Nicotinic acid	10 " "

The results from these studies are shown in table 1. It is apparent that the vitamin A content of the blood plasma of these calves was low at the beginning of these experiments. There was improvement in their general health and a reduction in the number of cases of scours as the result of feeding small amounts of shark liver oil. The vitamin A content of the blood rose sharply as the result of vitamin A feeding and continued throughout the period that the calves were under observation. The rise in blood plasma A was accompanied by a rise in the ascorbic acid in those calves which were deficient in vitamin C at the beginning of the experiments.

The vitamin B complex members which were given had an immediate and dramatic effect upon the calves' diarrhea. With the addition of the B complex in the presence of adequate vitamin A the feces returned to a normal consistency within 12 to 24 hours. Mortality in these calves was reduced to zero. Calves subsequently dropped in these herds were treated either with colostrum or shark liver oil and a week or two week's supply of the B complex given shortly after birth. No symptoms of scours developed. The preliminary data with the Jersey calves indicated that nicotinic and pantothenic acids were the members of the B complex which were the effective ones.

Some 8 to 12 cases of mild to severe calf "scours" in the University herd have since been treated with equal success. One case in particular is worthy of mention. A bull calf placed on a ration of mineralized whole milk containing Fe, Cu, Mn and Mg for a year developed stiffness. With the onset of stiffness a watery diarrhea began which continued for 4 or 5 weeks. This became increasingly worse. B complex capsules were administered at twice the rate given to the young calves. The diarrhea completely disappeared within 6 days with a marked improvement by the end of the third day.

These studies raised the question as to the blood plasma content of vitamin A and C of the new-born calf. As many newly dropped calves as could

TABLE 1

Effect of vitamin A upon the progress of calf scours and certain blood constituents

Calf	Vitamin A (gamma/cc.)						Carotene (gamma/cc.)			Ascorbic acid (mgs./100 cc.)		
	1/8/41	Scours	2/12/41	Scours	4/24/41	Scours	1/8/41	2/12/41	4/24/41	1/8/41	2/12/41	4/24/41
Holstein*												.31
76	.09	Severe	.21	Nil	.14	Nil	.17	—	.13	—	—	.61
47	.09	"	.18	Mild	.23	"	.26	.28	.66	.18	.29	.26
44	.08	"	.17	"	.21	"	.17	.23	.32	.20	.32	.22
45	.10	"	—	Nil	.16	"	.41	—	.76	.33	—	.49
9	—	"	.19	"	.16	"	—	.49	.72	—	—	.52
39	—	"	.14	Mild	.21	"	—	.14	.15	—	.26	
	3/14/41		3/27/41		4/24/41		3/14/41	3/27/41	4/24/41	3/14/41	3/27/41	4/24/41
Jersey†												
65	.10	Severe	.26	Nil	.09	Nil	.31	.37	.12	Trace	.40	.28
85	.08	"	.28	"	.14	"	.19	.29	.04	"	.26	.32
93	.07	"	.20	"	.14	"	.18	.13	.09	.42‡	.21	.37
95	.10	"	—	"	—	"	.87	—	—	Trace	—	—
84	.19	Very slight	.19	"	.13	"	—	.04	.05	"	.68	.23
92	.16	"	—	"	.18	"	.18	.13	.09	"	—	.34

* Shark liver oil 1/8/41 → 2/12/41, shark liver oil + B-complex 3/27/41.
† Shark liver oil.
‡ New-born calf.

be obtained before suckling were bled and the vitamin A, C and carotene content determined. The results are summarized in table 2. It is evident

TABLE 2

Summary data on the average vitamin A, C and carotene values of the new-born calf

Age	No. calves	New-born	No. calves	1 day	No. calves	2 days	No. calves	1 week	No. calves	3 weeks	No. calves	6 weeks
Vitamin C (mgs./100 cc.)	15	.53	4	.22	4	.29	8	.26	4	.35	5	.30
" A (γ/cc.)	16	.04	4	.14	3	.19	9	.18	5	.16	5	.18
Carotene (γ/cc.)	18	.05	4	.15	4	.20	7	.21	3	.13	5	.16

that the blood ascorbic acid is extraordinarily high in the new-born calf. It is also evident that the vitamin A content of the blood was far below normal, and in the region where a vitamin A deficiency develops (2). By the end of 24 hours the vitamin C dropped about 50 per cent and the vitamin A values rose some 3 to 4 times its original value, thus both moved toward and into the normal range. If the calves were segregated into groups depending upon the ration of their dams, there was some evidence which showed that calves from cows on average winter rations carried 0.04 gamma of vitamin A per cc. as compared to 0.08 gamma per cc. for the calves from dams fed extra quality hay and silage with their winter ration. The data further indicate that the new-born calf was amply fortified with ascorbic acid and that it ingested and obtained large amounts of vitamin A from colostrum milk. Thereafter the blood plasma values for these constituents remained normal under average conditions.

DISCUSSION

These observations again emphasize that the liberal use of colostrum in feeding calves is good management since it supplies the early requirement for vitamin A in the calf.

These studies show that the growing calf benefits from the administration of certain members of the vitamin B complex. This may account for the results obtained by Newman and Savage (7), who showed that brewers yeast in calf starters promoted greater growth in the young calf. Bechdel and associates (1), Wegner *et al.* (10) and McElroy and Goss (5) have shown that the ruminating bovine obtains large amounts of the known members of the vitamin B complex through the activity of bacteria in the rumen. In the calf fed milk only, the rumen does not seem to provide optimum conditions for synthesis of the B complex or else the requirements of the growing calf exceed the ability of the bacteria to synthesize the necessary amounts. It would seem likely that the rumen in the early life of the calf is not suffi-

ciently developed for the establishment of an adequate bacterial flora for the synthesis of the B complex.

On the basis of this work further studies on the nutrition of the new-born calf are being made.

CONCLUSIONS

These studies indicate that the calf diarrhea encountered was largely nutritional in origin. The administration of high vitamin A potency shark liver oil and certain members of the vitamin B complex eliminated the diarrhea and the resulting mortality from pneumonia. Preliminary evidence would suggest that nicotinic and pantothenic acids may be the factors of the B complex which were lacking.

New-born calves were found to be amply fortified with ascorbic acid but they were uniformly deficient in vitamin A. The ingestion of colostrum milk rich in vitamin A quickly brought about normal blood plasma levels. The ration of the dam only slightly influenced the amount of vitamin A found in the blood plasma of the new-born calf. Winter rations tend to reduce it while rations with ample carotene or fortified with vitamin A tend to raise it. Low ascorbic acid values in the blood plasma were increased by feeding shark liver oil rich in vitamin A.

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ESTIMATION OF INITIAL LIVE WEIGHT AT EACH LACTATION OF DAIRY COWS[1]

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For the purpose of this paper it is accepted that an estimate of live weight of the cow should be an integral part of each lactation record in all milk recording work. With reference to D.H.I.A. work, and other systems based on periodic visits of a tester, there are advantages to the routine in which the tester estimates the live weight of each cow that has calved since the last test of the particular herd. In connection with the subsequent lactation record of the cow, this estimate is designated as initial live weight. Such a record of initial live weight will serve the most important needs with a minimum of effort for the tester—a necessary consideration in view of the multitude of detail he has to look after.

The primary object of this paper is to develop and present a method of estimating initial live weight by use of a linear measurement of chest girth. Ragsdale, Brody, Davis, Morgan (1, 2, 3) have heretofore published tables for estimating live weight in pounds (W) of female dairy cattle from chest girth in inches (G) based on application of the equation, $W = aG^b$, to a total of 15,610 pairs of girth-weight measurements, in a large number of subdivisions by age and breed of animal. The observations were made at the Missouri and Nebraska Stations.

In the present paper, the Nebraska observations are reexamined from the special point of view of estimating initial live weight (initial weight in distinction to final weight, average weight, miscellaneous weight, undefined weight). Ample evidence has accumulated showing that initial live weight is a very important datum in the biological consideration of milk production problems. This paper proceeds from the foundation of the previous work cited to develop the detail of a plan of estimating initial live weight of each cow at each lactation, adapted to practical use in D.H.I.A. and other milk recording work.

Received for publication June 28, 1941.

[1] This paper is the result of a cooperative arrangement between the Nebraska and Illinois Stations, whereby the chest-girth and live-weight measurements made at the Nebraska Station over the past 19 years have been utilized at the Illinois Station to derive a formula for the purpose of estimating live weight from chest girth at one particular stage of lactation, namely, within the first 31 days after calving. Printed with the approval of the Director of the Nebraska Agricultural Experiment Station as Journal Series 292; and with the approval of the Director of the Illinois Agricultural Experiment Station.

EXPERIMENTAL OBSERVATIONS AND ANALYTICAL PROCEDURE

The observations used in the present paper were made at the Nebraska Station, from 1922 through 1940. Measurements of chest girth and live weight (and many other items not here utilized) were made at approximately the middle of each calendar month for the 19 years indicated on cows in the Nebraska Station herd. At the Illinois Station the girth-weight data have been transcribed to punched cards, one card for each lactation, starting with the first observation after calving in each lactation and continuing month by month thereafter as long as the succession was not interrupted up to and including the tenth month after calving. As the data are used this is the limit of capacity for a single card. As indicated, the primary concern of this paper is with the first observation after calving, to estimate initial live weight.

From the tables previously cited, it is known that both age and breed affect the girth-weight relationship. For example, a mature Holstein weighs about 200 pounds more than a 2-year-old Jersey of the same chest girth. Scrutiny of the tables and other considerations suggest age distinctions, sufficient for practical purposes, as follows: Cows under 3 years of age (< 3 yrs.); cows 3 or 4 years of age (3, 4 yrs.); cows 5 years of age or older (5, + yrs.). Age is reckoned as of date of measurement, that is, the age increases with advance in lactation are recognized.

Study of the previous tables cited suggested the possibility of combining the many equations in a single one, $W = a\,(G + g)^b$, in which g is an age-breed girth modifier, and still retain the accuracy of weight estimate associated with the age-breed distinctions. Indeed, if there were a single, known, true value for b, the single new equation using this true value of b, would be better than the many old equations, for general application. That is, while any one of the old equations is the best fit to the particular set of girth-weight observations from which it is derived it is not the best general equation for other similar observations if its b differs from the true value of b assumed to exist. It might be assumed from geometrical considerations (volumes of similar solids vary as the third power of corresponding linear dimensions) that the true value of b is 3. The old equations show, however, that the exponent 3 does not hold within age-breed groups. It may be doubted, therefore, that there is any single true value of b, valid for all age-breed groups. Nevertheless, it may be possible to select a single compromise value for b, applying satisfactorily to all age-breed groups, for the practical purpose of estimating initial live weight. If such a satisfactory value of b can be selected it is a simple matter to find the rest of the new equation, numerically. Our first step is to find the value of b from the observations of girth and weight for each of the age-breed groups for the first month of lactation.

Three formulas which may be used to compute the value of b in the equation, $W = aG^b$, are as follows:

1. $b_1 = \dfrac{n\,[S\,(\log G)(\log W)] - (S \log G)(S \log W)}{n\,S\,(\log G)^2 - (S \log G)^2}$

2. $b_2 = \dfrac{SG^{2b}\,[S\,(G^{2b})(\log G)(\log W)] - [S\,(G^{2b})(\log G)]\,[S\,(G^{2b})(\log W)]}{SG^{2b}\,[S\,(G^{2b})(\log G)^2] - [S\,(G^{2b})(\log G)]^2}$

3. $b_3 = \dfrac{[nSGW - (SW)(SG)]/SW}{[nSG^2 - (SG)^2]/SG}$

The first formula is the usual way of computing b. It minimizes $S\,(\log W_o - \log W_c)^2$ or $S\,[\log (W_o/W_c)]^2$, where W_o is observed W, and W_c is W computed by the fitted equation. It is the correct least-squares procedure, if we speak in terms of logarithms of the observations and not the observations themselves, and if G is considered to be without error.

The second formula, from Deming (4, page 147 and corrected page 143) minimizes $S\,(W_o - W_c)^2$. It is the correct least-squares procedure, if we speak in terms of the observations themselves (not their logarithms) and if G is considered to be without error. Since b itself is involved in this formula a necessary first step is to choose a value for b which is not too far from the b which is finally found by formula 2. This first approximation of b may be gotten by any method that gives a fair approximation. To hold G^{2b} within bounds any constant multiplier may be introduced, such as .00001.

The third formula is an approximation derived as follows. A straight line, $W = a' + b'G$, is fitted to the observations by least squares. The slope of this line is b'. The slope of $W = aG^b$ is baG^{b-1} and this is taken at the mean girth ($\overline{G}$) to be the same as that of the straight line. Then $ba\overline{G}^{b-1} = b'$, or multiplying through by $\overline{G}$, $ba\overline{G}^b = b'\overline{G}$. Next it is taken that the fitted power equation gives a value of W at the mean girth ($\overline{G}$) which is the same as the observed mean weight ($\overline{W}$), whence $a\overline{G}^b = \overline{W}$. Substituting and transposing, $b = b'(\overline{G}/\overline{W})$. In formula 3, SG/SW is substituted for $\overline{G}/\overline{W}$. The remainder of the formula, the part in square brackets, is simply a least-squares expression for b'. The formula is thus set up for efficiency in machine computation (including the electrically controlled punched card machines) dealing directly with the observations, and avoiding logarithms.

It is presumed that formula 3 may be used safely where the amount of curvature involved in $W = aG^b$ is not too great. Among the groups with which we are here dealing, the Jersey < 3 years records may be expected to involve the greatest amount of curvature. As a test of the applicability of formula 3, all 3 formulas have been applied to the 68 pairs of girth-weight measurements of this group, with the results: $b_1 = 1.799$; $b_2 = 1.795$; $b_3 = 1.789$.

If we are concerned with a least-squares solution of b in terms of the observations themselves (not their logarithms) we find, in this particular

case, b = 1.795. Accordingly, formula 1 is to be regarded as an approximation which gives b = 1.799, an error of 4 in the third decimal; while formula 3, frankly an approximation at the outset, gives b = 1.789, an error of 6 in the third decimal.[2]

TABLE 1

Value of b in the equation, $W = aG^b$ (heavy face), and number of pairs of girth-weight measurements (light face) according to breed, age, and month of lactation

Month	Ayrshire			Guernsey			Holstein			Jersey		
	< 3 yrs.	3, 4 yrs.	5, + yrs.	< 3 yrs.	3, 4 yrs.	5, + yrs.	< 3 yrs.	3, 4 yrs.	5, + yrs.	< 3 yrs.	3, 4 yrs.	5, + yrs.
1	**2.07**	**2.23**	**1.02**	**2.01**	**1.99**	**2.22**	**1.61**	**1.82**	**1.98**	**1.79**	**1.71**	**1.87**
	61	53	11	34	26	2	112	126	79	68	71	22
2	**2.23**	**1.80**	**1.50**	**2.05**	**1.84**	minus	**1.82**	**1.89**	**1.86**	**1.96**	**2.00**	**2.26**
	55	50	9	32	26	2	104	125	76	65	67	20
3	**2.08**	**2.09**	**1.61**	**2.10**	**2.11**	minus	**1.91**	**2.23**	**1.89**	**1.84**	**1.89**	**2.55**
	52	47	9	30	26	2	95	126	76	61	63	21
4	**2.43**	**2.19**	**2.25**	**2.07**	**2.28**	.....	**1.91**	**2.31**	**1.69**	**2.14**	**1.99**	**2.36**
	51	43	9	27	25	1	77	132	77	58	58	23
5	**2.17**	**2.36**	**2.40**	**2.21**	**1.78**	**0.31**	**1.93**	**2.17**	**1.55**	**2.33**	**1.90**	**2.20**
	47	42	8	24	23	2	61	140	74	55	59	22
6	**2.51**	**2.33**	**2.93**	**2.26**	**2.23**	**1.38**	**2.08**	**2.41**	**1.85**	**1.89**	**1.99**	**2.34**
	44	44	7	23	21	4	54	137	74	50	60	17
7	**..86**	**2.16**	**2.91**	**2.65**	**2.24**	**1.83**	**1.93**	**2.13**	**1.75**	**1.86**	**1.81**	**1.80**
	41	44	7	21	18	6	47	136	70	42	63	18
8	**1.75**	**2.49**	**2.75**	**1.74**	**2.35**	**2.64**	**2.13**	**2.12**	**1.83**	**2.02**	**1.86**	**2.03**
	37	41	9	18	19	7	37	133	75	40	59	19
9	**1.92**	**2.16**	**2.66**	**1.63**	**1.83**	**2.77**	**2.22**	**2.02**	**1.99**	**2.19**	**1.69**	**1.58**
	29	41	12	13	24	4	27	133	73	35	56	25
10	**2.32**	**2.28**	**2.83**	**1.15**	**0.92**	**2.23**	**2.08**	**2.17**	**1.75**	**2.21**	**1.64**	**1.85**
	22	45	10	10	26	4	17	131	76	23	63	19
1, 2	**2.14**	**2.01**	**1.22**	**2.03**	**1.89**	**1.58**	**1.71**	**1.86**	**1.93**	**1.85**	**1.83**	**2.05**
	116	103	20	66	52	4	216	251	155	133	138	42
3, 4	**2.25**	**2.14**	**1.97**	**2.07**	**2.20**	minus	**1.91**	**2.27**	**1.78**	**1.98**	**1.93**	**2.45**
	103	90	18	57	51	3	172	258	153	119	121	44
5, 6	**2.30**	**2.35**	**2.65**	**2.24**	**1.92**	**1.27**	**2.01**	**2.28**	**1.70**	**2.05**	**1.94**	**2.25**
	91	86	15	47	44	6	115	277	148	105	119	39
7, 8	**1.82**	**2.31**	**2.82**	**2.22**	**2.29**	**2.10**	**2.02**	**2.12**	**1.79**	**1.94**	**1.83**	**1.90**
	78	85	16	39	37	13	84	269	145	82	122	37
9, 10	**2.11**	**2.21**	**2.76**	**1.39**	**1.27**	**2.46**	**2.15**	**2.10**	**1.86**	**2.20**	**1.67**	**1.67**
	51	86	22	23	50	8	44	264	149	58	119	44
All 10	**2.19**	**2.24**	**2.36**	**2.11**	**1.91**	**1.95**	**1.89**	**2.13**	**1.83**	**2.04**	**1.83**	**2.08**
	439	450	91	232	234	34	631	1319	750	497	619	206
All 10 (all ages)		**2.32**			**2.23**			**2.10**			**2.08**	
		980			500			2700			1322	

[2] In the present case all 3 formulas give essentially the same result, presumably because the amount of curvature involved in the power equation is not great. If, however, all ages from birth to maturity (*e.g.*, 2, fig. 5) with the associated wide range in size of animal are thrown together, formulas 1 and 2 may give (and in the instance cited do give) very different values of b. (Incidentally, a similar situation may develop in expressing "basal metabolism" as a power function of live weight when animals of widely varying live weights, from "mouse to elephant," are thrown together.) Relatively speaking, formula 1 is more sensitive to deviations at low values of G and formula 2 is more sensitive to deviations at high values of G.

For present purposes it seems that the use of formula 3 is fully warranted, to avoid the extra labor involved in using formulas 1 or 2, applied to the individual pairs of girth-weight measurements. Formula 3 has been used to compute the value of b for each of the several age-breed groups by stage of lactation and the values of b thus derived are given in table 1, together with the respective frequencies. In figure 1 the b values are plotted against stage of lactation to bring out any trends in b with advance in lactation.

INITIAL LIVE WEIGHT EQUATION

The values of b in figure 1 show more or less distinct trends with advance in lactation. For purposes of estimating initial live weight it appears desirable, therefore, to deal with observations at an early stage of lactation, rather than to include all 10 months. On the other hand, the greater the number of observations the more reliable the resulting equation, other things being equal. A compromise is made by using the first two months of lactation in deriving an initial weight equation.

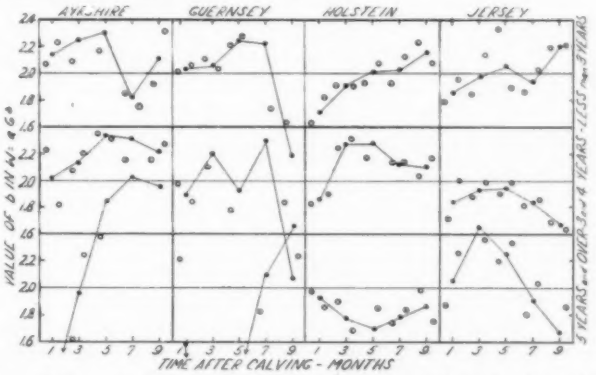

FIG. 1. Values of b in the equation $W = aG^b$ by stage of lactation for each of the age-breed groups. The solid circles connected by straight lines represent two-month periods. The open circles represent one-month periods, but do not include values which fall outside the limits 1.6–2.4.

From the data of table 1 and figure 1, it is concluded that the compromise, $b = 1.85$, is fairly applicable to all groups, for the particular purpose of estimating initial live weight. From table 2 the Jersey < 3 years group has an average weight of 819 pounds and average girth of 67.1 inches and a is computed as $a = 819/(67.1)^{1.85} = .342$. As a general equation, we now have $W = .342\ (G + g)^{1.85}$ where $g = 0$ for the Jersey < 3 years group. The girth-weight table of figure 2 is derived from this equation.

For the Jersey 3,4 years group the average weight is 922 and the average girth is 69.6. The $(G + g)$ value corresponding to 922 in figure 2 is 71.5 and

TABLE 2

Average chest girth, $\overline{G}$, in inches and average live weight, $\overline{W}$, in pounds

Breed	Age	Month or months of lactation					
		First one		First two		First ten	
	years	$\overline{G}$	$\overline{W}$	$\overline{G}$	$\overline{W}$	$\overline{G}$	$\overline{W}$
Ayrshire	< 3	71.2	976	71.2	970	71.6	1003
do	3, 4	74.0	1104	73.7	1092	74.4	1121
do	5, +	76.1	1135	76.2	1132	76.3	1164
Guernsey	< 3	70.0	928	70.1	931	71.0	968
do	3, 4	74.5	1073	74.2	1077	74.6	1108
do	5, +	75.6	1206	76.4	1198	76.1	1177
Holstein	< 3	76.6	1214	76.4	1208	76.2	1215
do	3, 4	80.1	1362	79.7	1348	79.5	1341
do	5, +	81.7	1431	81.4	1421	81.4	1424
Jersey	< 3	67.3	819	67.1	819	67.4	840
do	3, 4	69.8	923	69.6	922	69.7	930
do	5, +	72.6	995	72.3	976	72.1	984

accordingly the g value for this group is $71.5 - 69.6 = 1.9$, or 2 as given in figure 2. The value of g for each of the other age-breed groups is computed in a similar way. The device of figure 2 thus affords a compact table for estimating initial live weight from a chest girth measurement, recognizing age-breed groups.[3]

DISCUSSION

The diagram of figure 3 is presented to explain in more detail the principles involved in the present girth-weight equation. In it log W is plotted against log G as a straight line for each of two groups. It is clear that if these two lines have the same slope (the same value of b in the girth-weight equation) they may be made to lie on a common straight line by shifting the horizontal scale; likewise for any number of group lines of the same slope. It is the function of g in the equation to take care of this horizontal shift. The principle involved is rigorously valid if a single known value of b applies to all groups. It may be practically valid by using a compromise value of b, as in the present treatment.

If b is chosen too large the equation will overestimate live weight at large girths and underestimate it at small girths. If b is chosen too small an opposite situation will prevail. From table 1 and figure 1 it appears that the observed b is not larger than 1.85 for the initial data of the Holstein and

[3] Obviously age-breed differences in the girth-weight relationship must have an anatomical basis. The question arises, can g in the equation be based on some measurable dimension index (such as spring of rib) instead of age-breed, thus putting the weight estimate on a more accurate basis with respect to the individual cow? If such a dimension index is used to determine g the observations should be classified by this index (instead of age-breed) to determine the equation. That is, the index should be substituted for age-breed all the way through. The present use of age-breed is regarded as an expedient to be used until it can be replaced by a better system.

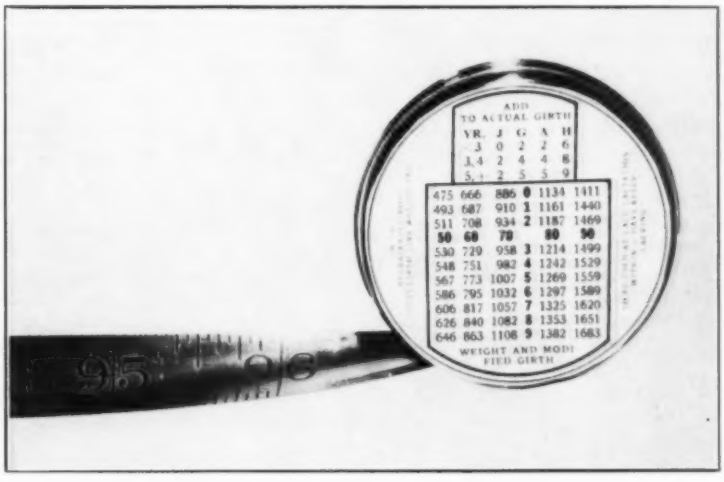

FIG. 2. Photograph of 96-inch steel tape rule and table for estimating initial live weight of cow from her chest girth. The tape has a)-shaped blade, $\frac{1}{2}$ inch wide. In operation the blade is withdrawn and the case end dropped over the back of the cow, thus flexing the blade which permits reaching the case from underneath the chest and completing the chest-girth measurement. (The blade does not flex freely under its own weight.) The tape is drawn under a force of about 3 pounds to encircle the chest in the region immediately back of the shoulders or region of smallest girth, and the girth read to the closest $\frac{1}{2}$ inch. This actual girth is then modified by addition of the age-breed factor indicated and the corresponding weight, in pounds, is read from the table, interpolating for the half inch, where necessary. *The table is not to be used until the actual girth has been modified as indicated.* Preliminary evidence indicates that the girth modifiers for Holstein cows should be used also for Brown Swiss and Milking Shorthorn cows. The tape pictured is a stock article of trade, except for the printed matter. It would be possible to incorporate the table on the blade of the tape, replacing the standard inch graduations with the appropriate weight figures, and supplementing the first end of the blade with an extension to take care of the girth modifier feature, thus providing a direct-reading girth-weight tape adapted to breed and age of cow.

Jersey breeds. The Ayrshire and Guernsey initial data suggest the exponent 2.00 rather than 1.85. If the present observations are representative of these two breeds the table of figure 2 (based on the exponent 1.85) will tend to overestimate weights at small girths and underestimate weights at large girths. The magnitude of this bias (if 2.00 is the correct exponent) is given by the fraction $[(G+g)/(\overline{G}+g)]^{.15}$. At the mean girth there is no bias; at $G+g=70$ and $\overline{G}+g=80$, which would be an extreme case, the correct (exponent 2.00) weight is .98 $[=(70/80)^{.15}]$ of the weight by figure 2 (exponent 1.85). That is, the weight is overestimated by 2 per cent at this point.

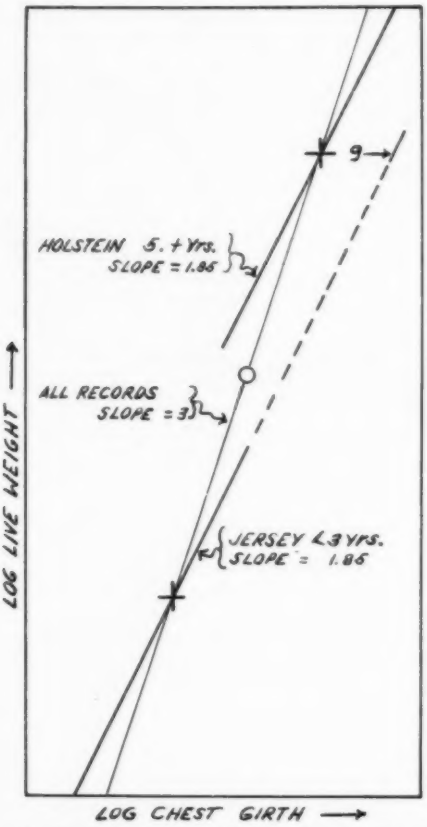

FIG. 3. Diagram to illustrate the principles involved in the girth-weight equation, $W = a(G+g)^b$. After selecting the compromise, $b = 1.85$, g is taken as zero for the Jersey < 3 years group and a is computed to make the equation line go through the observed mean girth and weight (cross) for this group. The observed mean weight (cross) of the Holstein 5,+ years group is then shifted onto the equation by the term, g, and other groups are treated in a similar way. Any group may have an observed b (table 1) somewhat different than 1.85 and hence can be shifted onto the equation at only one point. The observed mean weight is used as this point.

If all groups are thrown together the regression turns on the total mean girth and weight (circle) and tends to go through or close to the group means (crosses). In the diagram it has a slope of 3 which is about what is found in actual lumped observations. This is in conformity with the 3d power rule relating linear dimensions and volumes. On the basis of the experimental evidence of the total data and the geometrical considerations what could be more natural than to say that the 3d power rule is a general law relating girth and weight? But we have seen such a law does not hold within groups—a striking demonstration that the regression within groups may be quite different than the regression in total. The lesson may be taken into many other data, for example, if "basal metabolism" is related to live weight including species from "mouse to elephant" a beautiful "$\frac{3}{4}$ power rule" is revealed. One should be on guard about presuming that the same rule holds within species.

In a previous paper (5) live weight was estimated by the so-called New York tape, which is supposed to apply without regard to breed, age, stage of lactation, etc. The origin of this scale is unknown to the writers, but it conforms quite closely to a power equation with exponent 2.85. The difference between the scale of the New York tape and that of the Nebraska-Illinois tape, figure 2, is very serious as the following numerical comparison shows:

Chest girth, inches		50	60	70	80	90
Live weight, New York tape, pounds		388	656	1055	1502	1951
Live weight, Neb.–Ill. tape, (fig. 2), pounds	Holstein; < 3 years	586	795	1032	1297	
	Holstein; 5, + years		863	1108	1382	1683
	Jersey; < 3 years	475	666	886	1134	
	Jersey; 5, + years		708	934	1187	1469

As compared with the present scale, figure 2, the scale of the New York tape grossly overestimates the weight of large cows and grossly underestimates the weight of small cows. The magnitude of this bias may amount to 20 per cent or more.

A similar fault is bound to occur in any single scale derived from mixed breed, age, stage-of-lactation data. The fault arises in the fact that the regression of weight on girth is distinctly different within groups from what it is in total. This is especially prominent with respect to breed groups, less so with respect to age groups. The difficulty may be appeased by use of separate equations or by use of girth modifiers as in figure 2. Hopefully it may be overcome along the line suggested in footnote 3.

SUMMARY

An equation is derived for estimating initial live weight (live weight within 31 days after calving, in distinction to live weight at other or mixed stages of lactation) from chest girth. The equation is $W = .342\,(G+g)^{1.85}$, where W is initial live weight in pounds, G is actual chest girth in inches and g is a girth modifier for age and breed of cow, being zero for Jersey cows under 3 years of age and ranging up to 9 for Holstein cows 5 years of age or older. The equation permits a compact girth-weight table which is imprinted on the case of a steel tape rule used to measure the chest girth. As compared with the present girth-weight scale the scale of the New York girth-weight tape grossly overestimates the weight of large cows and grossly underestimates the weight of small cows, as much as 20 per cent. This fault of the New York tape is inherent in any formula derived from mixed data, with respect to breed, age, stage of lactation, and gestation.

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VOLUME XXIV NOVEMBER, 1941 NUMBER 11

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

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ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

782. Repeatability of Type Ratings in Dairy Cattle. LESLIE E. JOHNSON AND JAY L. LUSH, Iowa State College, Ames, Iowa.

Ratings for type in the Iowa State College Holstein-Friesian herd according to methods intended to be like those of the official voluntary type classification and extending over an eight-year period were examined for repeatability and other characteristics. The average repeatability was .34 among ratings by experienced judges who did not know the herd. This is about the same as the usual intra-herd repeatability of production records.

Ratings made before the animals were one year old were less reliable than later ones but there was little increase in repeatability after the animals had passed one year of age.

Ratings in consecutive years were but little more alike than ratings separated by two or more years.

Changes in the udder and in the general health of the cow appeared to be the chief causes of large shifts in the rating.

783. The Effect of Natural Milk Enzymes, Acid, and Salt upon the Keeping Quality of Butter Stored for Six Years. B. J. SCHEIB, C. N. STARK AND E. S. GUTHRIE, New York State Dept. Agr. and Markets, Albany; and Cornell University, Ithaca, N. Y.

See JOURNAL OF DAIRY SCIENCE, *24*, No. 6: 545. June, 1941, for abstract.

784. Superheated Soft Curd Milk. J. L. DIZIKES AND F. J. DOAN, Pennsylvania Agr. Expt. Sta., State College, Pa.

A method of producing soft curd milk by condensation, heat coagulation and homogenization, having a curd tension value of zero and digestion characteristics approximately equal to evaporated milk, is described. This product has been designated "superheated soft curd milk." When properly prepared it will exhibit digestion characteristics (*in vitro*) closely approaching those of human milk. It would require no boiling in the home and would offer fluid milk dealers, equipped to make it, a fresh milk product capable of competing with evaporated milk as far as digestibility is concerned.

785. Various Oils and Fats as Substitutes for Butterfat in the Ration of Young Calves. T. W. GULLICKSON, F. C. FOUNTAINE AND J. B. FITCH, Division of Dairy Husbandry, University of Minnesota.

Feeding tests were conducted to compare the feeding value for calves of the following fats and oils: butterfat, lard, tallow, coconut oil, peanut oil,

corn oil, cottonseed oil and soybean oil. The effect of a fat-poor diet also was determined.

A total of 47 young calves was used ranging in age from 6 to 29 days when placed on experiment. Their average age at start was 12 days. Each oil or fat was added to skim milk, homogenized to form a product containing 3.5 per cent fat and fed along with a low fat content concentrate mixture, cod liver oil, and usually some alfalfa hay.

One control group was fed normal whole milk, not homogenized. Test periods ranged from a few days to about six months in length.

In average daily gain in weight as well as in general appearance and well-being, the calves fed butterfat excelled those in all other groups. Following closely were those receiving lard and tallow. Corn oil, cottonseed oil and soybean oil were the least satisfactory. The average daily gains of calves in the various fat fed groups were as follows:

Butterfat	1.30 pounds
Lard	1.17 pounds
Tallow	1.24 pounds
Coconut oil	.96 pounds
Peanut oil	.80 pounds
Corn oil	.40 pounds
Cottonseed oil	.31 pounds
Soybean oil	.32 pounds

The calves in the three latter groups appeared unthrifty, listless and emaciated. Some calves in these groups died and others were saved only by changing to whole milk or reducing the amount of the oil fed.

Post-mortem examinations showed considerably more fat deposited in calves fed butterfat than in those receiving other oils or fats.

786. Further Studies on the Use of Salt for Improving the Quality of Cream for Buttermaking. F. E. NELSON, W. J. CAULFIELD AND W. H. MARTIN, Kansas Agr. Expt. Sta.

Laboratory and farm studies showed that the placing in the container at the beginning of seven- and ten-day collection periods of sufficient salt to give a concentration of 10 or 13 per cent in the fat-free serum of the cream at the end of the collection period resulted in marked improvement of the quality of the cream. Butter made from such cream or from cream to which the same amounts of salt had been added to the total amount of cream before a ten-day holding period at 70 or 82° F. began graded from one to five points higher than butter made from cream handled under the same conditions with no salt added. Less protein degradation (as shown by formol titration) and less development of acidity occurred in salted cream than in unsalted. The organoleptic grade on salted cream always was "sweet" or "first," whereas the grade on unsalted control cream always was at least one

grade lower than that of the corresponding salted cream and frequently was two grades lower. The salt affected the microflora of the cream both qualitatively and quantitatively. Maximum total bacterial counts of salted cream were considerably below those of unsalted cream. Acid-forming bacteria grew but little in the presence of the salt. Development of yeasts and molds appeared to be prevented completely. Large paired and clumped cocci and a few sarcina constituted the dominant flora of creams to which salt had been added. Salted cream apparently is non-corrosive to stainless steels of the types tested. Dairy metal and tinned copper were found to be subject to noticeable corrosion by salt-containing cream and probably would not be suitable materials for equipment used in collecting and processing such cream.

Addition of salt to cream offers an apparently feasible method for improving the quality of much of the butter manufactured in certain areas. Acceptance by regulatory officials and by creamerymen must be obtained before the method can be placed in the hands of the cream producer.

787. A Proposed Score Grade Method of Determining the Quality of Milk. P. A. Downs, University of Nebraska, Lincoln, Nebraska.

A new method of determining the quality of milk by scoring is proposed which will follow very closely the standards and ideas set forth in the Hand Book of Official United States Standards for quality of creamery butter.

This proposal is the beginning effort to unify the grading or scoring of the four (4) major products with a maximum and minimum score at a single level. As far as possible it is hoped that terms for all major dairy products can be simplified as suggested in this proposed scoring grade method of determining the quality of milk.

788. Size of the Rabbit Mammary Gland with Successive Lactations. A. A. Lewis and C. W. Turner, Univ. Missouri, Columbia, Mo.

It was thought that the rabbit might illustrate the influence of mammary gland development in dairy cows as a cause of the increase in milk production with succeeding pregnancies. This increased production is greater than can be accounted for by the increase in body weight. The lateral extension of the mammary glands in succeeding lactations was compared in rabbits with lines tattooed in the skin at the lateral extent of the mammary glands early in the first lactation. In eleven succeeding lactations only one case was found in which the mammary glands on one side extended past the tattoo line. The lateral extent of the glands did not increase in the other cases.

789. The Reversibility of Oxidative Inactivation of Milk Lipase in Relation to Its Activity in Cheddar Cheese. I. Hlynka and E. G.

HOOD, Science Service, Department of Agriculture, Ottawa, Canada.

The effect of three different reducing systems on oxidized milk lipase was studied. The lipase was inactivated by aeration alone or aeration and addition of copper. The systems were: (1) the endogenous reducing systems of deaerated milk, (2) ascorbic acid, (3) cysteine. The relation of these systems to those in cheddar cheese and of the inactivation of lipase to rancid flavour is discussed.

Portions of the same milk were aerated, aerated-deaerated (cysteine or ascorbic acid added before or after), and deaerated. The milk was incubated for 4 days at 5° C. and the extent of lipase activity was determined by titration of the fat phase.

Milk lipase was protected from oxidation by the exclusion of air while aeration and 0–8 p.p.m. Cu destroyed it. Deaeration of aerated milk restored some of the activity of oxidized lipase but deaeration of aerated milk to which Cu had been added did not. Ascorbic acid gave inconclusive results. Cysteine showed definite reversibility of oxidatively inactivated lipase with or without the addition of copper.

BACTERIOLOGY

790. Differential Staining of Living and Dead Yeast Cells. D. R. MILLS, Oregon State College, Corvallis, Ore. Food Res., *6*, No. 4: 361. July-Aug., 1941.

Methylene blue, methyl green and erythrosin were found to give a sharp distinction in staining between living and dead yeast cells. A solution containing 1: 10,000 methylene blue is recommended for differential staining. The authors state that plates do not give representative counts of the number of living cells in a fermenting culture but merely the number of reproducing cells, while the proposed staining method produces a rapid and accurate estimate of all viable cells. F.J.D.

791. A Study of the Germicidal Action of Ultraviolet Light. I. Use of Ultraviolet Rays in Vegetable Hydrocoolers. F. R. SMITH AND R. L. PERRY, Univ. California, Davis, Calif. Food Res., *6*, No. 4: 345. July-Aug., 1941.

The portion of this study of possible interest to the dairy industry was that wherein liquids were treated with ultra-violet light radiations from lamps submerged in the liquids. This treatment increased the germicidal effectiveness of the lamps with turbid liquids. At low temperatures, however, the activity of the light was diminished due to a decrease in the energy coming from the lamps. F.J.D.

BOOK REVIEWS

792. Textbook of Bacteriology. EDWIN O. JORDAN AND WILLIAM BURROWS. Thirteenth Edition. W. B. Saunders Co., Philadelphia. 1941. 731 pages, illustrated. Price $6.00.

The present edition of this book represents a complete revision of the previous edition. The first 11 chapters deal with general bacteriology. Two chapters are devoted to immunity and the following 23 to pathogenic microorganisms, their morphology, physiology, and various characteristics of medical significance. Additional chapters discuss the virus, virus diseases of man, bacteriophage and the Rickettsiae.

One new departure carried out in this edition is the elimination of chapters devoted to highly specialized fields such as soil, industrial and dairy bacteriology and their incorporation with other materials into a chapter on bacterial physiology. Such an approach undoubtedly will find favor with the instructor who emphasizes bacteriology from the medical standpoint and is interested in the great number of industrially important microorganisms only because of their purely scientific significance. This arrangement may limit somewhat the value of the book to those who in their teaching desire to emphasize equally the medical and industrial phases of microbiology.

This book is intended primarily for students interested in the medical aspects of bacteriology and in this respect is well written and up to date. Water and milk and other foods are considered primarily from the standpoint of their importance in transmission of disease. The subject of immunity, including recent knowledge on immunochemistry and the nature and medical significance of the filterable virus, is discussed in detail. Where subject reviews are available, the author has attempted to supply these and where such reviews are not available the discussions in the text have been expanded to briefly review them. Numerous references are supplied for statements cited in the discussions. P.R.E.

793. Biological Stains. H. J. CONN. Fourth Edition. Biotech Publications, Geneva, New York. 1941. 308 pages. Price $3.40.

The fourth edition of this book maintains the high standard set in the three previous editions. A careful revision has been made to bring all statements up to date and to correct any errors occurring in the other editions.

Thirteen new dyes are included among which are chlorazol black E, important as a general purpose stain, and the series of thiazol dyes now being employed in fluorescent microscopy.

For the information of those not familiar with earlier editions of this book it is sponsored by the Commission on Biological Stains, an independent organization affiliated with the National Research Council. Included are chapters on the history of staining, general nature and classification of dyes,

the spectro-photometric analysis of dyes and theory of staining. Six chapters are devoted to discussions of individual dyes and related groups of dyes. The discussions include chemistry of the dyes and the suitability of the dyes for various biological purposes.

Other useful information is supplied in a series of tables that list important stains, their nomenclature, synonyms, application and bibliographic references. Methods for evaluating dyes as biological stains are also described. Numerous references to original articles are provided. P.R.E.

BREEDING

794. Achondroplasia in Calves. GRAYDON W. BRANDT, Ohio State University, Columbus, Ohio. Jour. Hered., *32*, No. 6: 183–186. 1941.

The literature dealing with achondroplasia in cattle is reviewed and two cases of achondroplasia are reported which are believed to be of the recessive type. J.L.L.

BUTTER

795. What Is Your Part in the National Quality Campaign? H. C. DARGER, Amer. Butter Inst., Chicago. Natl. Butter and Cheese Jour., *32*, No. 9: 14. 1941.

Improvement of butter quality in the future will depend on cream quality which is indicated to a limited extent by tests for mold mycelia in cream and butter. Cream quality campaigns are necessary; the most effective plan is to educate creamery managers and procurement supervisors in a state-wide meeting so that they in turn can hold district meetings. Buyers meetings can be organized in the same way. This organization should be maintained by the industry. Creamery men can cooperate financially, also by grading and by observing the three principal factors in producing and marketing quality cream, namely, Sanitation, Time, and Temperature. Judicious use of the mold mycelia test can be helpful in educating patrons. W.V.P.

CHEESE

796. Valuable Information on Process Cheese. C. R. BARKER. Natl. Butter and Cheese Jour., *32*, No. 8: 14. 1941.

Uniformity of flavor and consistency are attained by intelligent blending, critical cleaning, and trimming, coarse to medium grinding, controlled heat treatment, thorough stirring and protective packaging. The base or 70 per cent of a batch is "short-held" cheese, one to three months old and "storage," six months held cheese. This cheese should be smooth and buttery, not sticky or crumbly, and two-thirds should be on the "acid-side" but not acid while one-third should be sweet. Short-held cheese tends to

make a firm, tough body while storage cheese softens the body. About 15 per cent of the batch should be "current" cheese, tough body, while 12 to 15 per cent can be "acid" cheese. The blend is most apt to be successful when cheeses from 5 to 7 factories, preferably from different sections of the United States, are used. Cheese should be tempered to 70° F. in 72 hours before blending. Emulsifiers, such as sodium citrate, di-sodium phosphate, sodium potassium tartrate, glyconates, ortho-, meta-, and poly-phosphates and di-basic citric acid salts and a caseinate are used at the rate of 1 to 2 per cent of the weight of the cheese; too much may make a stiff, coarse body. Overrun caused by the addition of emulsifier, salt and water usually equals 5 per cent for American cheese and higher for other varieties. The heating process pasteurizes and prepares the cheese for packaging. Transparent wrappers are displacing foil. W.V.P.

797. Factors Affecting Quality of Cheddar Cheese. S. L. TUCKEY, Univ. Illinois, Urbana. Natl. Butter and Cheese Jour., *32*, No. 9: 12. 1941.

Bacterial content, acid development, moisture and salt are important factors affecting flavor of Cheddar cheese. Decomposition of the protein is of prime importance but recent experiments indicate that hydrolysis of fat and percentage of salt in the cheese also contribute to flavor development. The influence of salt was investigated by making ten lots of cheese. The curd was salted and, after 20-, 40- and 60-minute intervals, portions were removed from the vat and pressed. In the meantime the salty whey draining from the curd was poured back over the curd. Lots of cheese salted 60 minutes were superior to those salted for shorter periods because they scored higher in flavor, contained more salt, and were never criticized for bitter flavor. When the salt content was 1.7 per cent or more, ripening was delayed and the cheese body was "curdy." Prolonging the salting period beyond 60 minutes incorporated more salt and decreased the moisture. The scores indicate that cheese to be aged should contain more salt than cheese to be marketed fresh. Changes in cheese fat constants including iodine, saponification, Reichert Meissl, Polenske and acid numbers were followed for a year. The acid number showed the most significant changes indicating hydrolysis of fat. Lipase action was used to stimulate fat hydrolysis by adding homogenized raw cream to pasteurized skimmilk. A rancid flavor was produced that persisted in the cheese for at least 16 weeks. Experiments to study ripening changes are being continued. W.V.P.

798. Determining Volatile Acids in Cheese. J. C. MARQUARDT AND A. C. DAHLBERG, New York Agr. Expt. Sta., Geneva, N. Y. Natl. Butter and Cheese Jour., *32*, No. 8: 40. 1941.

Procedure for estimating volatile acids in hard and semi-hard cheeses

have been studied and a preferred standardized method is described. A 3000-ml. round bottom flask is used for generating steam. This is connected to a 2000-ml. flask for the cheese sample and a 400-ml. Liebig condenser; all side arm and flask connections are ground glass unions. The procedure involves filling the 3000-ml. flask with 2500 ml. of distilled water. The cheese is mixed with 400 ml. of water and 25 ml. of 25 per cent H_2SO_4 solution. Distillation proceeds until twenty 100-ml. portions of distillate are collected. Size of sample is regulated so tha' between the fifteenth and twentieth aliquots the distillate becomes neutral to deci-normal NaOH. Sample sizes allowing "complete distillation" in the twenty aliquots are: young cheddar—200 gms.; aged cheddar—100 gms.; young semi-hard (45 per cent H_2O or more)—200 gms.; and cured semi-hard—25 gms. The total deci-normal NaOH required to neutralize the aliquots of distillate from 200 gms. of cheese is called the "volatile acid equivalent." The procedure can be used in standardizing flavor in process cheese or cheese foods and is applicable in studying changes in curing cheese. W.V.P.

CHEMISTRY

799. Promoting the Oxidation of Fats and Oils. Relative Effectiveness of Different Bands of the Visible Spectrum. GEORGE R. GREENBANK AND GEORGE E. HOLM, Bur. Dairy Indus., U. S. D. A., Washington, D. C. Indus. and Engin. Chem., Indus. Ed., *33*, No. 8: 1058. 1941.

The effectiveness of different light bands in promoting oxidation of corn oil, cottonseed oil, lard and butter oil increases with a decrease in the wave lengths used, energy from the blue end of the spectrum being most effective. The light bands were obtained with the use of filters and a monochromator, and were adjusted to equal intensity. The amount of light absorbed in the yellow, orange and red regions of the spectrum was almost constant for each oil and only in lard did it exceed 5 per cent. Light absorption and amount of peroxides formed increased progressively as the wave length of light decreased from the yellow, the amount of peroxides produced being dependent upon the type of oil used. B.H.W.

800. Microbiological Assay for Pantothenic Acid. F. M. STRONG, R. E. FEENEY AND ANN EARLE, Univ. Wisconsin, Madison, Wis. Indus. and Engin. Chem., Analyt. Ed, *13*, No. 8: 566. 1941.

A method for the determination of pantothenic acid has been developed which utilizes the need of *Lactobacillus casei* E for this substance. A medium essentially free from pantothenic acid but otherwise adequate for this organism is used. The lactic acid produced during growth of the organism in a medium containing the unknown is determined by direct titration

and the quantity of pantothenic acid calculated by means of a standard curve. Investigation has shown the method to be reliable. The pantothenic acid content of a number of biological materials including several dairy products is given. B.H.W.

801. Application of the Refractometer to Determination of Total Solids in Milk Products. V. D. LUDINGTON AND E. W. BIRD, Iowa State College, Ames, Iowa. Food Res., *6*, No. 4: 421. July-Aug., 1941.

Studies revealed that the total solids of skimmilk at various stages of condensation and dilution can be determined refractometrically with sufficient precision for commercial usage, standard deviations indicating that the measurements on which calculations are based are accurate. The use of ammonium hydroxide in the skimmilk showed no advantage.

Refractive indices of milk and cream cannot be determined with accuracy due to the dispersion of light by the fat emulsion. Satisfactory readings, however, can be obtained in samples to which 60 per cent sucrose is added but the variations in the calculated total solids are too great to make the procedure commercially feasible. No advantage was found in reading the samples at 40° C. (125° F.) as compared with 25° C. (77° F.). F.J.D.

DISEASE

802. Sodium Chlorate Poisoning in Cattle. G. R. MOORE, Dept. Surgery and Med., Kansas State College. Jour. Amer. Vet. Med. Assoc., *99:* 50. 1941.

Sodium chlorate ($NaClO_3$) according to the author has been widely recommended in recent years as a means of eradicating quack grass and bindweed. The author is of the opinion that this type of poisoning in cattle and swine may be more common than is generally suspected. Causes and symptoms are described. Total dosage is discussed together with diagnosis and treatment. Prevention is suggested as the best remedy, consisting of: 1. Salt the herd thoroughly before taking any chance with them on chlorate sprayed ground. 2. Avoid using the spray in dry form, especially avoid piles on the ground. 3. Remove empty containers. F.E.

803. Further Investigations of Rumen Gases and Bloat in Ruminants. R. W. DOUGHERTY, Oregon Expt. Sta., Corvallis, Ore. Jour. Amer. Vet. Med. Assoc., *99:* 110. 1941.

An interesting group of experiments is reported in which the H_2S present in rumen gases is correlated with the type of feed ingested. The greener and higher quality the feed the more H_2S was found in the rumen gas samples. The amount of carbon monoxide (CO) was also determined, but its occurrence was found to be similarly correlated with the type of feed in-

gested. The amount of H_2S required to produce prostration varied greatly when introduced through a rumen fistula. Operative, sampling and analyses techniques are described. F.E.

804. Studies in Brucellosis. I. F. HUDDLESON, W. B. ARDREY, R. B. PENNELL, W. H. STAHL, E. E. HAMANN, AND MYRTLE MUNGAR. Mich. Agr. Expt. Sta. Tech. Bul., 177. 1941.

This report comprises a series of nine papers. Subjects investigated were: 1. A study of factors influencing the isolation, cultivation, and differentiation of the species of *Brucella*. 2. The presence of a capsule on Brucella cells. 3. Study of cross skin sensitization between *Pasteurella tularensis* and *Brucella mellitensis*. 4. The immunizing value of the gluco-lipid antigen from *Brucella* cells. 5. Conditions for maximum precipitation and agglutination of antibody in various *Brucella* antiserums. 6. The presence of *Brucella* antibody in the urine of human beings. 7. Separation and study of the lipid fraction of *Brucella abortus*. 8. A study of the leucocytic picture in brucellosis. 9. A study of the effect of the toxic fraction from *Brucella* cells on the leucocytic picture in normal guinea pigs.

In these studies it was shown that more than 0.5 per cent peptone in the medium tends to retard growth of *Brucella*. A concentration of 1:700,000 of crystal violet was recommended when beef liver agar containing 0.5 per cent peptone is used in isolation. Bacto-Tryptose agar was highly satisfactory for the isolation and differentiation of the three species of *Brucella* in the presence of dyes.

Data indicated that the presence and amount of capsular material determined virulence of the cells. A special procedure for capsule demonstration was described. Results suggested that when the clinical diagnosis on humans is doubtful a skin test with protein nucleate solution from *Brucella* or *P. tularense* should serve as a means of differentiating brucellosis and tularemia. The gluco-lipid antigen from *Brucella* cells exhibited no immunizing value against experimental *Br. abortus* infection in guinea pigs.

To obtain precipitation of the maximum amount of *Brucella* antibody nitrogen for cow antiserum and endoantigen, two hours at 37° plus 22 hours at 4° C. were required. Forty-eight hours at 4° C. provided maximum removal of *Brucella agglutinin* antibody nitrogen for cow antiserum and homologous cells. *Brucella* antibody was demonstrated in the urine of certain brucellosis patients. The method for concentrating this antibody was described. The lipids which composed about five to six per cent of the cells were found to be biologically inactive.

Leucopenia with a relative lymphatocytosis and slight monocytosis characterized the blood picture in *Brucella mellitensis* infected individuals. Non-filamented neutrophiles were higher than normal. Pathologic lymphocytes with liver endothelial cells were found in 40 per cent of the cases. All

cases exhibited finally varying degrees of basophilic granulation of neutrophiles. The data indicated that the Brucella toxic fraction aided by tissues of the gastro-intestinal tract had a cytolytic effect on neutrophiles in the blood vessels. P.R.E.

FEEDS AND FEEDING

805. Comparative Feeding Value of Silages Made from Napier Grass, Sorghum and Sugarcane. A. L. SHEALY, W. G. KIRK, AND R. M. CROWN. Fla. Agr. Expt. Sta. Bul. 358. 18 pages. 1941.

Three feeding trials with steers were conducted. The same amount of concentrates, consisting of ground snapped corn and cottonseed meal, was fed to each lot together with as much of the respective silage as the steers would consume. In each of the three trials more sorghum silage was consumed than of either of the other kinds.

Sorghum silage contained more dry matter, crude protein, and nitrogen-free extract than did either Napier grass silage or sugar cane silage. Due to higher digestibility of its crude fiber and N-free extract the sorghum silage contained more T.D.N. than either of the others did.

	Sorghum silage	Napier grass silage	Sugar cane silage
Average daily gains (lbs.)	2.08	1.81	1.79
T.D.N. required per lb. of gain (lbs.)	6.16	6.30	6.27
Value for fattening steers on a comparative basis	100	75	70

J.G.A.

806. Growth and Development. III. Relation between Organ Weight and Body Weight in Growing and Mature Animals. S. BRODY AND H. H. KIBLER. Mo. Agr. Expt. Sta. Res. Bul., 328. 41 pages. 1941.

This bulletin should be a valuable reference for workers in animal physiology.

Detailed charts are presented relating the weights of each of the major visceral organs to the corresponding body weights in (1) mature mammals of different species; (2) mature birds of different species; (3) animals of the same species, growing and mature. The relative-growth equation $Y = aX^b$ was fitted to each set of data and the numerical values of the exponent, b, discussed with reference to the metabolic levels. For mature animals of different species, the weights of the neuro-endocrine systems, such as the brain and pituitary gland, which are the metabolism-controlling organs, tend to increase with approximately the same fractional power as does basal metabolism; the cardio-respiratory systems, such as the heart, which carries

the working burden of the body, tends to increase more directly with body weight than with the basal metabolism. The organ-body relations in growing animals vary with the stage of growth; the value of b tends to be lower for later growth in the same species than for mature animals of different species. The results are discussed from theoretical and practical view points.

A list of 50 references is given. J.G.A.

807. Condensed Whey as a Profit Item. J. EDWARD TUFFT. Natl. Butter and Cheese Jour., *22*, No. 8: 11. 1941.

Whey condensed to 35 to 50 per cent solids and placed in 50, 500, and 600 pound drums is being sold at a profit by a California creamery to poultrymen and feed mills. W.V.P.

808. Distribution of "Trace Elements" in the Newborn Calf as Influenced by the Nutrition of the Dam. L. L. RUSOFF. Fla. Agr. Expt. Sta. Bul. 359. 47 pages.

The tissues and organs of three newborn calves from normal dams and one newborn calf from a "salt sick" dam were analyzed for trace elements by a spectrographic estimation.

Of the 27 trace elements for which the various organs and tissues in this study were analyzed, 15 were detected, namely: aluminum, barium, boron, chromium, cobalt, lead, manganese, molybdenum, nickel, silver, strontium, tin, titanium, vanadium and zinc. The following elements were not detected in any of the tissues or organs: arsenic, antimony, beryllium, bismuth, cadmium, cesium, lanthanum, lithium, thorium, tungsten, yttrium, and zirconium.

Zinc was found in all of the tissues and organs which were examined; aluminum and manganese were detected in practically all of the tissues; barium, lead, molybdenum and strontium in about one-half of the tissues; nickel and silver in about one-third, and boron, chromium, tin, titanium, cobalt and vanadium were found sporadically.

A comparison of the distribution and concentration of trace elements in the tissues and organs of newborn calves from normal and from "salt sick" dams showed no significant differences.

Thirty-two references; 12 tables, 5 of which are in the appendix are given. J.G.A.

809. Distribution and Concentration of Copper in the Newborn Calf as Influenced by the Nutrition of the Dam. L. L. RUSOFF. Fla. Agr. Expt. Sta. Tech. Bul. 356. 61 pages. 1941.

A comparison of the copper content of the tissues usually analyzed, of four newborn calves, as influenced by the nutrition of their dams, was made by a quantitative spectrographic method.

A comparison of the copper content of all tissues and organs of two of the newborn calves, one from a normal dam and one from a "salt sick" dam, also was made.

Copper was present in every organ and tissue analyzed.

The entire normal newborn calf contained 236.6 to 237.0 milligrams of copper and the entire "salt sick" newborn calf contained 269.9 to 271.2 milligrams.

With the exception of the skeletal tissues, practically all of the "salt sick" tissues and organs contained more copper than corresponding normal organs and tissues.

The hypothesis that bone may be a site for copper storage is supported.

The dam's being "salt sick" does not influence copper storage in the newborn calf.

No definite conclusions can be drawn concerning the role of copper in "salt sick." More biological work needs to be done before a physiological interpretation of the metabolism of copper in "salt sick" can be given.

A calf from a "salt sick" dam is not itself "salt sick."

Eighty-two references; 28 tables, 7 of which are in an appendix to the bulletin, are given. J.G.A.

810. The Value of Urea in the Synthesis of Protein in the Paunch of the Ruminant. I. In Maintenance. L. E. HARRIS AND H. H. MITCHELL, Univ. Illinois, Urbana. Jour. Nutr., *22:* 167–182. 1941.

Sheep fed a basal ration low in protein were on negative nitrogen balances. These negative nitrogen balances were always improved when either casein or urea was incorporated in the ration. The data showed that sheep may be maintained in nitrogen equilibrium for more than 100 days on rations containing urea and minimal amounts of protein providing only one-tenth of the amount of nitrogen needed for equilibrium. At nitrogen equilibrium the biological value of the urea nitrogen was 62 and of casein nitrogen 79. Urea improved the digestibility of cellulose markedly.

C.F.H.

811. The Value of Urea in the Synthesis of Protein in the Paunch of the Ruminant. II. In Growth. L. E. HARRIS AND H. H. MITCHELL, Univ. Illinois, Urbana. Jour. Nutr., *22:* 183–196. 1941.

The addition of urea to a low-nitrogen ration which was in itself unable to support appreciable growth in lambs or even consistently to maintain nitrogen equilibrium converted it into a ration capable of promoting a normal or nearly normal rate of growth. Tests with casein indicated a clear superiority of casein nitrogen at levels of 15 per cent protein equivalent over the urea nitrogen at either 15 or the 11 per cent protein equivalent level. Rations containing 3.16 per cent urea on the dry basis were not toxic.

C.F.H.

812. The Vitamin A Requirements for Normal Growth in Young Dairy Cattle. H. T. CONVERSE AND E. B. MEIGS, U.S.D.A., Washington. Amer. Soc. Anim. Prod. Proc., *32:* 67–72. 1939.

Calves were fed from the fourth day of age a low vitamin A basal ration supplemented with varying amounts of carotene ranging from 29 to 87 μg. per kg. body weight and similar levels of vitamin A ranging from 7.55 to 22.65 μg. per kg. body weight. On both the highest and lowest levels calves died or developed subnormally, and the results indicated that the optimum was an amount approximately twice the lower level. At this age the requirement was larger per unit of body weight than for ages 6 to 24 months.

G.C.W.

813. Carotene Requirements of Dairy Cattle for Reproduction. A. H. KUHLMAN AND W. D. GALLUP, Oklahoma A. and M. College, Stillwater. Amer. Soc. Anim. Prod. Proc., *33:* 67–73. 1940.

Using a basal ration of cottonseed meal and prairie hay it was found that a minimum of 40–45 μg. of carotene per pound of body weight was required daily by Jersey cows during the 270 days preceding parturition to produce normal calves, and to start a normal lactation. G.C.W.

814. High-phosphorus vs. Low-phosphorus Red Clover Hay for Growing Calves. H. R. DUNCAN, Univ. Tennessee, Knoxville. Amer. Soc. Anim. Prod. Proc., *33:* 109–111. 1940.

The only difference in the ration of calves each receiving 6 lbs. of clover hay, ground yellow corn, salt and cod liver oil was the P content of the hay, being 0.116 per cent and 0.309 per cent, respectively. In 196 days the average gain in weight was 79 lbs., in height at withers 2.21 inches, and in heart girth 2.46 inches for the low P group compared to 124 lbs., 3.46 inches and 5.42 inches, respectively, for the high P group. The cost of a given amount of gain was 33 per cent more for the low P fed animals, and they were decidedly less thrifty. The low P hay was grown under circumstances assumed to be favorable on a farm much above the average in productiveness.

G.C.W.

815. Further Studies of the Influence of Fat Intake on Milk and Fat Secretion. L. A. MAYNARD, J. K. LOOSLI AND C. M. MCCAY, Cornell Univ., Ithaca, New York. Amer. Soc. Anim. Prod. Proc., *33:* 340–344. 1940.

Two recent trials, one continuous and one double reversal, comparing grain rations of 7 per cent and 3 per cent fat, respectively, bear out earlier results in this laboratory of increased milk and fat percentage yields on the higher fat rations. The several trials (10) show increased daily yields of

fat-corrected milk ranging from 0.27 to 3.54 lbs. per cow. It is not known whether fat *per se*, or some fatty acid, or some other component of ether extract has been responsible. G.C.W.

FOOD VALUE OF DAIRY PRODUCTS

816. The Effect of Certain Fats and Unsaturated Fatty Acids upon the Utilization of Carotene. W. C. SHERMAN, Lab. of Anim. Nutr., Ala. Agr. Expt. Sta., Auburn. Jour. Nutr., *22:* 153–165. 1941.

The effect of supplementing a diet low in fat and carotene with linseed oil, corn oil, wheat germ oil, butterfat, cottonseed oil, methyl linolate and methyl linolenate on carotene utilization was investigated with rats. Soybean oil, cottonseed oil, linseed oil, corn oil, and wheat germ oil had a beneficial effect upon growth. Butterfat and coconut oil had no appreciable effect. Methyl linolate and methyl linolenate had an antagonistic action when fed with low levels of carotene which was counteracted by the addition of soybean oil and also by feeding carotene and methyl linolate a few hours apart. C.F.H.

817. Butter and Nutrition. ALICE COOLEY, Natl. Dairy Council, Chicago. Natl. Butter and Cheese Jour., *32*, No. 9: 16. 1941.

There are three basic reasons why a home-maker should buy butter. First, it is a dependable source of vitamin A since it is eaten three times daily and because the vitamin A in butter is better utilized than carotene, the form in which the vitamin occurs in fruits and vegetables. Vitamin A builds resistance to infection and a deficiency of it causes "night-blindness" and eventually xerophthalmia. Second, butter contains a growth-promoting factor other than vitamin A. Data show the superiority for growth in young animals of butterfat over such vegetable oils as corn, cotton seed, soy bean and cocoanut although the known fat soluble vitamins, such as A, D, and E, were adequately provided. Third, our people use less than half as much butter as other English-speaking people, yet there is no substitute for butter. Flavor and digestibility of butterfat should commend its use. Fortification of other foods with vitamins requires more research to disclose the importance of butter in the diet. W.V.P.

818. Further Experiments on the Calcium Requirement of Adult Man and the Utilization of the Calcium in Milk. F. R. STEGGERDA AND H. H. MITCHELL, Univ. Illinois, Urbana. Jour. Nutr., *21:* 577–588. 1941.

Twenty-five calcium-balance periods of 12 to 32 days each were carried out on nine adult men. The basal diet which furnished 203 mg. of calcium daily was supplemented with milk products (liquid whole milk, liquid skim

milk, "dried milk solids" and homogenized milk) in amounts to provide enough calcium for approximate equilibrium. The calcium in the different milk products studied was utilized at about the same degree of efficiency. An average utilization of 29 per cent was observed. The average calcium requirement for equilibrium was 9.55 ± 0.46 mg. daily per kilogram of body weight. C.F.H.

819. **Pasteurization and the Nutritive Value of Milk.** C. A. ELVEHJEM, Univ. Wisconsin, Madison, Wis. Dairy World, *20*, No. 3: 18. Aug., 1941.

This paper is a very good summary of recent studies and recent thought relative to the effect of heat on the nutritive properties of milk. The author does not confine himself merely to comparisons of pasteurized and raw milk but touches on seasonal variations, higher heat treatments, exclusive feeding of milk to animals through more than one generation and several other interesting related subjects. In conclusion he states that, "we are unable to find on a nutritional basis any objection to the production and use of pasteurized milk." F.J.D.

820. **The Ascorbic Acid Content of Cows' Milk during Pregnancy.** A. D. HOLMES, F. TRIPP, E. A. WOELFFER AND G. H. SATTERFIELD, E. L. Patch Co.; H. P. Hood and Sons, Boston, Mass., and Univ. North Carolina, Raleigh. Jour. Nutr., *22:* 267–271. 1941.

The ascorbic acid content of the milk of Guernsey and Holstein cows was determined from time to time throughout pregnancy. The animals were stall fed. The ascorbic acid values showed considerable variation although they tended to decrease slightly with advanced pregnancy. C.F.H.

821. **Nutritive Value of Quick Frozen Foods.** FAITH FENTON, Cornell Univ. Refrig. Engin., *42*, No. 2: 140. 1941.

The vitamin C content of quick frozen vegetables was studied. In general, it was found that the vitamin C content was very high in quick frozen vegetables and much higher than that of fresh vegetables which have been held even a few hours at room temperature. The author cautioned against the use of too much water in cooking the vegetables and then discarding it because of the high degree of solubility of the vitamin. For the same reason frozen vegetables should not be thawed before cooking and they should be cooked in only a very minimum of water. L.M.D.

ICE CREAM

822. **A Study of Skim Milk for Ice Cream.** L. K. CROWE AND HARRY H. WINN, Univ. Nebraska. Ice Cream Field, *38*, No. 2: 40. 1941.

The authors discuss the results obtained in an investigation designed to

study the effects of freezing and storing upon (a) plain condensed skim milk, (b) superheated condensed skim milk and (c) sweetened condensed skim milk. A review is given of previously published work related to the problem.

It was found that fresh plain and sweetened condensed skim milk were almost completely soluble and only very slightly more soluble than fresh superheated condensed skim milk. The solubility of the plain condensed skim milk decreased slowly over the three months storage period, whereas the solubility of the superheated condensed skim milk decreased rapidly over this same period. The sweetened condensed skim milk was almost completely soluble after three months storage.

The results showed no consistent change in protein stability (alcohol number) of ice cream mixes made with condensed skim milk which had been stored frozen for one month when compared to mixes prepared with fresh condensed skim milk. It is stated "Mixes made with stored frozen superheated condensed skim milk showed less change than mixes prepared with plain or sweetened condensed skim milk." Protein stability of ice cream mixes made with each type of condensed skim milk stored frozen two months was greater than that of mixes made with fresh skim milk or condensed skim milk stored frozen for one or three months. No apparent relationship was found between the solubility of condensed skim milk and the alcohol number of ice cream mixes in which it is used.

There was very little difference in the titratable acidity of the ice cream mixes made with the three types of fresh condensed and the results indicated that freezing and storing condensed skim milk for periods up to three months did not increase the titratable acidity of ice cream mixes in which it was used. The pH of the ice cream mixes was always within the range 6.2 to 6.4.

Viscosity of ice cream mixes was not consistently affected by the freezing and storing of the three types of condensed skim milk used. Mixes made with fresh plain condensed skim milk whipped to 100 per cent overrun faster than ice cream mixes made with fresh superheated and fresh sweetened condensed skim milk. Mixes made with the three types of condensed skim milk stored frozen for one month whipped more slowly than those prepared from the corresponding fresh condensed skim milks. It is stated, however, that the time required to reach 100 per cent overrun in ice cream mixes made with the three types of condensed skim milk stored frozen was less than the time required for mixes made with fresh condensed skim milk.

There was no appreciable difference in the flavor scores of ice cream attributed to the type of condensed skim milk used whether fresh or frozen, but there was a tendency for the body and texture scores to be less as a result of using the three types of frozen condensed skim milk. Freezing and storing plain and superheated condensed skim milk increased the amount of foam, whereas the opposite was true for sweetened condensed skim milk.

Ice creams made with the frozen condensed skim milk were frequently criticized for flaky or curdy appearance upon melting. W.C.C.

823. Effects of Freezing and Hardening on Ice Cream Texture. J. H. ERB, Ohio State Univ., Columbus, Ohio. Ice Cream Field, *38*, No. 2: 46. 1941.

The author describes a laboratory experiment carried out in connection with a class in ice cream in which a given ice cream mix was frozen and hardened under four different conditions. The results showed that the body and texture scores varied as much as 2.5 points. The poorest ice cream was produced by drawing it from the batch freezer in a soft condition and hardening it slowly at 0° F. Ice cream taken from the same freezer and hardened more rapidly at – 30° F. showed an improved score of from 0.5 to 1.0 point. Stiff ice cream drawn from the batch freezer and hardened slowly was about equal in texture to the soft drawn ice cream which was hardened rapidly. The ice cream drawn from the continuous freezer at the same temperature as from the batch freezer showed a slightly better texture. The smoothest textured ice cream was that drawn from the continuous freezer at 22° F. and hardened rapidly. W.C.C.

824. Ice Cream and Problem of Rising Costs. JACK NISBET, Secretary, Ohio Assoc. Ice Cream Mfrs. Ice Cream Field, *38*, No. 2: 20. 1941.

It is pointed out that increased costs of ice cream ingredients have necessitated some increase in the wholesale price of ice cream. In some instances this has been used as a basis of relatively large increases in retail prices resulting in marked decreased consumption. It is suggested that it may be necessary to prepare suitable streamers and signs for "6¢ cones" and "12¢ sodas" to keep the retailer from increasing his price in 5¢ jumps.

The author states that the food value of ice cream should be stressed during the present emergency to avoid having people consider it a luxury. The advisability of correcting misconceptions is emphasized and several "mistaken ideas about ice cream" are cited. W.C.C.

825. Merchandising Ice Cream and Associated Products. CHARLES STICH, Kats and Besthoff, New Orleans, La. Ice Cream Field, *38*, No. 2: 28. 1941.

Attention is called to the fact that in 1931 Drug Stores accounted for 30.5 per cent of the nation's ice cream volume, whereas in 1938 it accounted for 28.6 per cent, while company-owned retail stores in 1931 accounted for 2.3 per cent of the national ice cream volume as compared with 5.7 per cent in 1938.

The author considers ice cream quality of prime importance and concludes that ice cream should have a high butterfat content, be rich, smooth and creamy and full of flavor. In addition it should have a descriptive name such as: velvet; all-cream; tru-flavor; flavor-fresh, etc.

Reference is made to a survey which shows that ice cream's greatest appeal—20 per cent—is quality, and its least appeal—2.3 per cent—is price. Ice cream appeals as a food to 15 per cent of the customers and as a refreshment to 12 per cent of them. He emphasizes the advantage of selling ice cream as a food as well as supplying suitable specials for various occasions throughout the year. Mention is also made of window displays as a means of merchandising ice cream.

The importance of "The carry-out ice cream business" is stressed and the increased sales resulting from the use of a special package for this purpose are cited. W.C.C.

826. Are There Profits in Pecan Ice Cream? EDWARD G. MARSH, R. E. Funsten Co., St. Louis, Mo. Ice Cream Field, *37*, No. 6: 26. 1941.

The author quotes the International Association of Ice Cream Manufacturers as showing by a survey that butter pecan accounts for 3.67 per cent of the U. S. ice cream production. He claims that if properly processed, pecans do not become soggy or woody when incorporated in ice cream.

Reference is also made to U. S. Department of Agriculture Bulletin 324 entitled, "An Economic Study of the Pecan Industry," which reports that if price is not a factor that consumer preference for pecans outranks their preference for all other nuts.

Shelled pecans are obtained primarily from the wild or seedling variety, whereas the papershell or improved variety is usually sold in the shell. It is stated that the pecan industry has 17 grades of shelled pecans from which the ice cream industry may select.

Various methods of introducing and mechandising pecan flavored ice creams are mentioned. W.C.C.

827. Do You Sell a 10¢ Pint? ANONYMOUS. Ice Cream Field, *37*, No. 6: 6. 1941.

This is a report of the findings of a survey by the Institute of Ice Cream Opinion which is under the direction of the Ice Cream Field. The results show that 86.9 per cent answered this question in the negative while 13.1 per cent of the replies indicated that their competitors sell a 10¢ pint. It was further revealed that 86.2 per cent of the replies favor a low priced package ice cream as a means of increasing gallonage and that the average of the price range given by these replies was 18.009¢.

There was considerable variation in different sections of the United States, with California and the South West areas showing a greater tendency to sell this low priced item than the Northern areas. W.C.C.

828. Getting the Most out of Flavors in Ice Cream. C. A. IVERSON, Iowa State College. Ice Cream Field, *38*, No. 1: 49. 1941.

It is stated that although the trend in the ice cream industry is towards more delicate flavors and colors and towards smoother textured ice cream that sudden changes even in the direction of the trends may cause consumer objection.

The author points out that serum solids have a depressing effect upon flavor in addition to any "off" flavors which they may carry. It is postulated that butterfat acts as a flavor carrier whereas serum solids to a certain extent act as flavor depressors.

In discussing "vacreation," a system of vacuum pasteurization, it is stated that from $\frac{1}{4}$ to $\frac{1}{3}$ less flavor is required to obtain approximately the same flavor intensity with vacreated mix as with mix pasteurized in the usual manner.

It is stated that it is not always possible to predict whether or not a given substance may serve as a satisfactory flavor in ice cream. It is actually necessary to try the combination in ice cream. It is suggested that caramel and fudge flavors be used in combination with chocolate and furthermore that mint flavor mixes well with chocolate.

Fresh ground coconut meat makes a very pleasing flavored ice cream. When used alone from 4 to 6 quarts of ground coconut is required for 10 gallons of finished ice cream. Coconut pineapple and coconut cherry also are pleasing combinations, it is stated.

Nuts for ice cream must have pleasing flavor and be crisp and tender in the ice cream. It is reported that dry roasting the nuts and treating them with butter previously heated to 330° F. is most satisfactory because it develops a roasted nut flavor, dries out the nut and the butter coating causes the nut to retain the crispness to a remarkable degree.

Butter is used at the rate of 1 to 2 pounds for 8 pounds of nuts.

Mention is also made of the importance of sanitation in handling and preparation of nuts. The heat treatment mentioned is a safeguard in this connection.

Pecans, English walnuts, black walnuts, almonds, and Brazil nuts are mentioned as satisfactory either alone or in various combinations. In addition hazelnuts or filberts, cashew nuts and pistachio nuts are mentioned as good possibilities. W.C.C.

829. How Can We Improve Our Sherbets and Ices? P. H. TRACY, Univ. Illinois. Ice Cream Field, *37*, No. 6: 36. 1941.

The author defines ices and sherbets according to the standard adopted by the International Association of Ice Cream Manufacturers and points out that in certain instances they play a relatively important role in the ice cream industry.

A list of seven desirable features of stabilizers is given for ices and sherbets. This is followed by a brief discussion of the more common stabilizers used including gelatin, various gums, pectin, and sodium alginate. The advantages and disadvantages of each stabilizer mentioned is briefly outlined and directions for its use are given.

The author recommends the use of monosaccharides—such as honey, dextrose or sweetose—in conjunction with cane or beet sugar to prevent surface crustation. It is stated that satisfactory results were obtained using 14 per cent sucrose and 21 per cent sweetose. The author also recommends from 0.35 per cent to 0.50 per cent citric acid content depending upon the sugar content of the mix.

Brief mention is also made of the following defects in ices and sherbets: bleeding, surface crustation, crumbly body, snowy body, coarse body, sticky body and ice separation.

A few suggested formulas are given. W.C.C.

830. Ice Cream Sales Soar. ANONYMOUS. Ice Cream Field, *38*, No. 2: 21. 1941.

A brief discussion is given of the statistical report of the United States Agricultural Marketing Service, and the Ice Cream Sales Index compiled by the Statistical and Accounting Bureau of the International Association of Ice Cream Manufacturers. It is pointed out that except for the second four-months period of 1936, the first four months of 1941 had the largest sales increase of any four-months period during the 16 years the Sales Index has been published. W.C.C.

831. Coffee as a Summer Flavor. ALEXANDER KATZ, Florasynth Labs., Pacific Coast Div. Ice Cream Field, *38*, No. 2: 34. 1941.

In a comparison of twelve flavors, coffee was selected as the outstanding flavor by the public and vanilla was considered second.

Extreme care is necessary to properly prepare concentrated liquid coffee flavor. It is stated that synthetic coffee flavor cannot replace the flavor prepared from natural coffee.

Many uses are mentioned for this product including the flavoring of ice cream, milk, milk shakes, candy, etc. W.C.C.

832. Fruits on the March to Greater Sales. VINCENT M. RABUFFO, Editor, Ice Cream Trade Jour., New York. Ice Cream Trade Jour., *37*, No. 7: 15. 1941.

The sale of fruit flavored ice cream has been increasing. Black and red raspberries, cherries, pineapples, and peaches are being used in ever increasing quantities. A knowledge of the characteristics of the various varieties

of fruits, how to pack and preserve them for ice cream, and the taste and eye appeal which fruit impart to ice cream, are factors responsible for their increased use. Amounts used, common varieties, sources and methods of preparation for use in ice cream are discussed in this article. W.H.M.

833. Profits the Milky Way. LOUIS D. JONES, Hamilton Beach Co., Racine, Wis. Ice Cream Trade Jour., *37*, No. 7: 12. 1941.

A description is given of how a successful merchandiser boosted his sale of ice cream by featuring ice cream and milk drinks. A creamy delicious malted milk or milk shake, an ice cream soda, tangy-zestful with flavor varieties to create wide taste appeal and a sundae with outstanding eye and taste appeal were used as leads. High quality ingredients, properly trained help, personal interest in his customers and accurate cost records are listed as some of the major points in his success. W.H.M.

834. Current Sales Trends. O'NEAL M. JOHNSTON, Washington, D. C. Ice Cream Trade Jour., *37*, No. 6: 39. 1941.

With the exception of January, May, August and September, every month in 1940 showed an increase in the sale of ice cream in the United States according to figures released by the Statistical and Accounting Bureau of the I. A. of I. C. Manufacturers, Washington, D. C. in Special Bulletin Number 65. The net increase for the year was 2.56 per cent above 1939 sales. W.H.M.

835. Stabilizing Water Ices and Sherbets. B. I. MASURVOSKY, Editor, Ice Cream Trade Jour. Ice Cream Trade Jour., *37*, No. 6: 35. 1941.

A replacement of 25 to 30 per cent of the sucrose in sherbets and ices with invert sugar, dextrose or corn sirup solids is suggested. There are several stabilizers for sherbets and ices and usually satisfactory results can be obtained by following the directions furnished with these products. Poor results sometimes occur because the composition of the mix is altered when fruits are added to the mixture. W.H.M.

836. Smoothness in Ice Cream. G. C. NORTH, Beatrice Creamery Co. Ice Cream Trade Jour., *37*, No. 6: 13. 1941.

Several factors, homogenization, proper balance of ingredients and faster freezing, have tended to eliminate coarseness in ice cream texture and a smoother product has resulted. Smoothness in texture is definitely related to size of ice crystals, size and number of air cells, presence of lactose crystals and butterfat particles. Homogenization increases the total fat globule surface and produces a lubricating effect making a given sample of ice cream appear to be smoother and the fat also offers mechanical obstruction to the formation of ice crystals.

By increasing the non-fat milk solids in the mix, the formation of large ice crystals is obstructed and the amount of water available for ice formation is reduced. The use of an excessive amount of total solids should be avoided, since freezing and storage difficulties may be encountered and defects such as sandiness and sogginess may result. A proper relationship between the fat and non-fat milk solids should be maintained and the kind and amount of sugar regulated to avoid excessive sweetness and freezing difficulties. Stabilizers and eggs also tend to improve the texture of ice cream.

W.H.M.

837. England's Ice Cream Industry under Fire. CLIFFORD SKERTCHLY, London, Eng. Ice Cream Trade Jour., *37*, No. 6: 10. 1941.

With the use of fresh milk and cream forbidden and the use of butter, concentrated milk products and sugar greatly restricted, ice cream manufacturers in England have turned to the use of substitute products. At first about the only change in the mix was the substitution of margarine for butterfat, later soya bean flour, corn flour and wheat flour replaced the milk solids. It also became necessary to reduce the sugar content from 13 per cent to 11 per cent and to substitute honey for part of the sugar. The author states that England's war-time ice cream seems to be palatable and its selling price shows no appreciable increase. The following is one of the recommended formulae used in England: 1 pound of fat; 8 ounces of wheat flour; 1 pound of sugar; 2 ounces of egg yolk; water to make 1 gallon.

W.H.M.

838. A New Quick Freezing System. L. H. BARTLETT AND H. E. BROWN, Bur. Engin. Res., Univ. Texas. Refrig. Engin., *42*, No. 2: 83. 1941.

The advantages and disadvantages of fluid contact freezing are described and the use of a polyphase freezing medium in order to overcome disadvantages of the liquid medium is explained.

This polyphase medium is a chilled solution of a freezing point depressing solute, through which is dispersed the solid phase of the solute in finely subdivided form. The mechanism of heat transfer is one largely of conduction. Decrease in the fluid film resistance of a polyphase medium is probably due to the fact that the thermal conductivity of ice is very much greater than that of the liquid phase, it being 1.3 btu. per ft. hr. °F. at 0° F., while that of a 63 per cent syrup is only 0.19 btu. per ft. hr. °F. at the same temperature. The weighted mean conductivity of a polyphase medium containing 5 per cent by weight of suspended ice in 63 per cent syrup is 0.27 btu. per ft. hr. °F., which is 1.38 times the conductivity of the liquid phase.

The polyphase medium removes a given quantity of heat from foods in

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The polyphase medium removes a given quantity of heat from foods in

about 60 per cent of the time required by a liquid medium of similar composition and at the same temperature.

The heat transfer rates are nearly independent of fluid velocities enabling very slow circulation rates to be used, thus reducing energy losses to the medium. Also very viscous media can be employed at low temperature.

The authors have developed a continuous freezing machine of simple design employing the polyphase medium of water-invert sugar refrigerated at −2° F. capable of freezing 50 lb. of product per hour, weighing 700 lb. and requiring only 8 ft. by 16 in. of floor space. The food product is fed into a hopper at one end. The circulation is maintained by the helicoid conveyor, and by varying its speed, contact-time may be varied from 5 to 17 min. Low power is required, less than ¼ h.p. being needed (exclusive of refrigeration) to operate the 50 lb. unit. Foodstuffs are frozen as individual pieces and thus may be stored in bulk containers from which they may be packed in smaller units as desired later. Because of the elasticity of the freezing fluid, delicate food products are not broken or crushed, while agitation is secured not only by means of the spiral motion in the helicoid conveyor but by means of a unique drive gear which rotates the screw about one-fifth turn forward and then about one-sixth turn backward. L.M.D.

MILK

839. The Filter Disc as a Sediment Tester on the Farm. JOSEPH BURNS, Schwartz Mfg. Co., Two Rivers, Wis. Natl. Butter and Cheese Jour., *32*, No. 9: 56. 1941.

It is suggested that the filter disc viewed as a sediment test by the farmer can stimulate the production of better milk. Filter discs and cellophane-covered cards on which to mount them were given to 8 farmers with the request that they return them at the end of a week to the plant receiving their milk. Plant technicians made daily methylene blue and sediment tests of each patron's milk. Inspection of the filter discs indicated that some patrons tried to keep the milk cleaner and the author believes that keeping dirt out of milk leads to other good practices. The tabulated results of the plant technicians' tests show no improvement in methylene blue or sediment during the six-day observation period. W.V.P.

840. Proposed A.S.R.E. Methods of Rating and Testing Complete Can-type Milk Coolers. The American Society of Refrigerating Engineers, New York, N. Y. Refrig. Engin., *42*, No. 3. 1941.

Methods of rating can-type milk coolers developed by a special committee of the American Society of Refrigerating Engineers. The general purpose of this code is to provide the purchaser with the standard ratings so that he may select adequate equipment insofar as capacity and speed of cooling are

concerned. These methods, it is believed, will result in rating information entirely understandable by the dairyman. The test procedures include only electrically driven mechanical units. The methods proposed consist of Definitions, Types and Accuracy of Instruments, Rating Tests, Calculation of Test Results, General Data to be Recorded, and Test Report. The two most important ratings to be determined for any cooler are the Rated A.S.R.E. capacity and Rated A.S.R.E. power consumption. Different testing procedures are employed for non-ice-making coolers and ice-making coolers. Formulas are given for calculation of the above ratings and for the A.S.R.E. Rated Milk Cooling Coefficient, the latter being watt hours per gallon per degree F. The committee recommends that the Rated A.S.R.E. capacity and power consumption be included as one point in the manufacturers' published capacity data. L.M.D.

841. Refrigeration Requirements in the Processing and Marketing of Milk and Milk Products. KENNETH M. RENNER, Texas Technol. College. Refrig. Engin. *42*, No. 2: 90. 1941.

A resume of literature dealing with refrigeration requirements in connection with milk production, market milk handling, and milk products manufacture. The author points out that the following factors should be considered in their effect on refrigeration requirements: 1. The type of product that is to be refrigerated, and the specific requirements with respect to temperature control of the product. 2. The methods employed in the processing of the product. 3. The peak load in any one day's operation. 4. Type of equipment being used in the plant. 5. Methods of marketing the product. L.M.D.

842. Marketing of Milk Products in Lenawee County, Michigan. ORION ULREY, Mich. State College Agr. Expt. Sta., East Lansing, in cooperation with the Bur. of Agr. Econ., U. S. D. A. Spec. Bul., 310. June, 1941.

This is a 42-page report of one segment of a study made in various states by the agricultural experiment stations in cooperation with the United States Bureau of Agricultural Economics. This report deals with the marketing of milk and butterfat in Lenawee County, Michigan, with special consideration being given to local and interregional competition. Production conditions also were studied on selected farms in two townships.

Main purposes of the investigation were to determine the types of local market outlets, the place of the county dairy plants in processing milk products, and the competitive position of the cream from this area in the eastern markets. Special attention was given to the inspection of cream by the city of Detroit and by the cities in the East.

An attempt was made to analyze the principal milk marketing problems of the area. One of the chief problems was found to be the lack of a uniform system of inspection of farms and plants by states and municipalities and a large variation in strictness of the enforcement of regulations. P.H.T.

843. Stability of Homogenized Milk in Cookery Practice. HERBERT HOLLENDER AND K. G. WECKEL, Univ. Wisconsin, Madison, Wis. Food Res., *6*, No. 4: 335. July-Aug., 1941.

Homogenized milk was found to be definitely less stable (more easily coagulated) in cookery practice than unhomogenized milk. In the preparation of escalloped potatoes the usual methods of stabilizing milk by adding sodium citrate, phosphate or carbonate produced variable results depending on the characteristics of the potatoes used. The acidity of the potatoes appeared not to be a measure of their effect.

In the preparation of cooked oatmeals or wheat cereals, homogenized milk produced a whiter and more creamy dish than did unhomogenized milk and the coagulation was either retarded or prevented by the use of table salt in amounts normally used for seasoning. Preheating the milk did not influence its stability in the cereals. The type and degree of water hardness also was found to be a factor influencing stability.

Homogenized milk used in egg custard caused a lower curd tension and greater serum separation; however, the use of a lower cooking temperature and a shorter period of time overcame the serum separation to a considerable degree. F.J.D.

844. Dairy Plant Refrigerating Systems. RAY B. WOLF, Borden Co., San Francisco, Calif. Ice and Refrig., *99*, No. 4: 234. 1940.

The importance of refrigeration to the fluid milk industry is pointed out by showing that out of a total milk production of 12,500,000,000 gallons in the United States, 3,875,000,000 gallons are used for fluid milk and cream of which amount nearly 45 per cent or 1,743,750,000 gallons are pasteurized. It takes 5.35 tons refrigeration to properly handle 1000 gallons of pasteurized milk, and only about one-half of this is supplied by water. About 12 per cent of the cooling load is before pasteurization, 43 per cent after pasteurization and the balance is used for storage rooms. In addition, $\frac{3}{4}$ to $1\frac{1}{4}$ tons of ice (the equivalent of $1\frac{1}{2}$ tons refrigeration) are needed for every 1000 gallons of product for delivery purposes.

The author points out that with the exception of very large plants, it is more profitable for a dairy to buy its ice than to manufacture it. The disadvantages of brine as a cooling media in modern dairy equipment are pointed out. Chilled water, and the flooded type of direct expansion are to be preferred. The size of the unit depends on the operating procedure,

but a 6 × 6 unit will very nicely take care of a 50-quart filter. It is advisable to have auxiliary refrigerating equipment in order to avoid costly shut downs if a break down occurs.

Different types of pasteurizing machinery are briefly touched upon. Proper controls are emphasized. L.C.T.

PHYSIOLOGY

845. Variations in Dairy Bull Semen with Respect to Its Use in Artificial Insemination. H. A. Herman and E. W. Swanson. Mo. Agr. Expt. Sta. Res. Bul. 326. 82 pages. 1941.

A comprehensive study, undertaken in order to determine the quality of semen produced by bulls whose breeding efficiency was known, to compare the actual efficiency of semen used in artificial insemination with the results of physical and chemical examinations of the sample, and to determine, if possible, the best measure of evaluation of semen to be used for artificial insemination of dairy cows under practical field conditions.

A critical examination of semen from 342 separate ejaculates representing 55 dairy bulls was made. The fertility of 51 of the bulls was determined from their breeding records and compared with the examination of their semen.

The semen samples were found to vary widely in all properties studied. The variation was observed in different ejaculates of the same bull as well as in ejaculates of different bulls. The variations in initial motility and pH were not great. The greatest variations observed were in the length of time vigorous motility persisted and percentage of abnormal spermatozoa.

The time of survival with good motility was found to be a good index of fertility of the semen. The longer vigorous motility was maintained in a sample of semen, the greater was the chance of that semen producing conception.

The pH, initial motility, and percentage of abnormal spermatozoa were found to be related to fertility of the semen to only a limited extent. Semen of high pH, 7.00 or higher, was usually of very low viability. Semen of poor initial motility survived only a short time. A normal pH, good initial motility and low percentage of abnormal spermatozoa were an indication but not assurance of good fertility.

Morphologically abnormal spermatozoa were found in every sample of semen examined. Bulls which produced semen averaging 30 per cent of abnormal spermatozoa were usually of poor fertility, but not all samples of semen containing over 30 per cent abnormal spermatozoa were infertile. The most common types of abnormal spermatozoa, in order of their occurrence, were coiled tails, tailless, and pyriform. In the semen observed in this study no one of the three main types of abnormal spermatozoa seemed of more significance to infertility than other types.

Survival time of semen which was normally of good quality was not increased by dilution with any of the common diluents used. Survival of semen from a few bulls was materially increased by dilution with egg-yolk-buffer dilutor. Such semen normally became thick and viscous in storage and the dilutor apparently prevented this or other deleterious effects.

Fertility of good quality semen was maintained from 3 to 5 days when stored undiluted at 40° F.

The fertility of a bull cannot be accurately estimated from a single semen examination. Three or more semen samples examined several days, or even weeks apart, with accompanying records of the bull's actual breeding record, provides the most accurate method of evaluating fertility.

A bibliography of 89 references is appended. J.G.A.

846. New Portable Equipment for Measuring Respiration of Sperm Cells. R. E. COMSTOCK, Univ. Minnesota, St. Paul, Minn. Amer. Soc. Anim. Prod. Proc., *33:* 216–220. 1940.

Apparatus is described for field measurement of respiration of semen samples. The principle is the same as for the Haldane gas analysis and the Bancroft apparatus (modified by Dixon) for direct measurement of volume change. In comparison with the Warburg method the correlation coefficient was .88. Corrections were made for temperature and barometric pressure. The equipment is portable and moderate in cost. G.C.W.

847. The Site of Elaboration of the Pituitary Gonadotropic Hormone and of Prolactin. MAURICE H. FRIEDMAN AND S. R. HALL, Bureau of Dairy Industry, U. S. D. A. Endocrinology, *29:* 179. 1941.

Beef pituitaries showed a much higher concentration of gonadotropic hormones in the central portion than in the peripheral portion. The reverse was true of prolactin although the difference was not so great. During a time interval when the rabbit pituitary was almost completely exhausted of its gonadotropic hormone content there was no significant change in the prolactin content. It was concluded that prolactin and the gonadotropic hormone were secreted by the rabbit pituitary at independently variable rates and that in both the bovine and the rabbit pituitary, prolactin and the gonadotropic hormone behave as separate and independent physiologic entities. R.P.R.

848. Progresterone-like Effect of Ascorbic Acid (Vitamin C) on the Endometrium. S. LEON ISRAEL AND D. R. MERANZE, Mount Sinai Hospital, Philadelphia. Endocrinology, *29:* 210. 1941.

Infantile rabbits and ovariectomized adult mice, rats and rabbits were primed by means of daily injections of estrogen for 7 days. The experi-

mental groups were then injected daily with monoethanolamine salt of ascorbic acid for 5 days. The control animals received either no further injections (estrogen controls) or daily injections of progesterone for 5 days (progesterone controls). The uteri of the 3 groups were then compared as to weight, gross appearance and microscopic characteristics. The progesterone controls developed the heaviest uterine horns, the estrogen controls the lightest and the vitamin treated animals were intermediate. Microscopically the endometrium of the vitamin treated animals approximated the secretory changes of the progesterone group. This response was most marked in infantile rabbits. R.P.R.

849. Variations in the Yield of Gonadotropic Material from Green Plants in Relation to the Season of Growth and the pH of the Fresh Juice. MAURICE H. FRIEDMAN AND JOHN W. MITCHELL. Bureau of Dairy and Plant Industry, U. S. D. A. Endocrinology, *29:* 172. 1941.

In the experiments conducted there was a seasonal variation in the yields of gonadotropic activity from the juice of immature Sudan grass, oat and corn plants. Inactive juices were obtained from plants in autumn, winter and spring and these were characterized by a high initial pH and a high yield of solids in the washed and dried benzoic acid precipitate. Active juices were obtained from plants in the summer months and these were characterized by a low initial pH and a low yield of solids in the precipitate. Most of the active samples, however, were of relatively low potency, averaging about 1/20 of the potency of the late pregnancy urine. R.P.R.

850. The Reproductive Organs in Malnutrition. Effects of Chorionic Gonadotropin upon Atrophic Genitalia of Underfed Male Rats. MICHAEL G. MULINOS AND LEO POMERANTZ, Dept. Pharmacology, Columbia Univ. Endocrinology, *29:* 267. 1941.

The effects of acute starvation and of chronic underfeeding on the testes and the accessory genitalia of the male rat were studied. During chronic inanition there was atrophy of the accessory sex organs and of the interstitial cells of Leydig. The loss in weight of the testes and the diminution in the size and spermatogenic activity of the seminiferous tubules were considerable. These regressive changes were corrected by the injection of chorionic gonadotropin. Small doses over a 40-day period being more effective than large doses over a shorter period of time. Likewise, inanition sterility was corrected by the injection of chorionic gonadotropin. Complete starvation for 7 days lowered testicular weight as well as the weight of the accessory genitalia, particularly that of the seminal vesicles. R.P.R.

851. Use of Stilbestrol in the Suppression of Lactation. SAMUEL D. SOULE AND A. R. BORTNICK, St. Louis, Mo. Jour. Clin. Endocrinol., *1:* 409. 1941.

A group of 50 postpartum women were treated with stilbestrol in an attempt to suppress lactation. Five mg. of stilbestrol were administered once, twice or three times. Breast engorgment was relieved or inhibited within 16 hours in 30 of 40 patients in the early puerperium. Treatment after the second week of puerperium, after lactation was well established, did not prove satisfactory. R.P.R.

852. Clinical Effect of Stilbestrol on Postpartum Activity of the Mammary Glands. A. W. DIDDLE AND W. C. KEETTEL, State Univ. of Iowa. Jour. Clin. Endocrinol., *1:* 494. 1941.

One hundred postpartum women were treated with stilbestrol for breast engorgment. Forty-six were multiparas and 54 primiparas. Treatment was continued from 1 to 6 days and the dosage varied from 5 to 50 mg. When the administration of stilbestrol was begun within 24 hours postpartum and continued for 5 days, 10 mg. daily, breast engorgment was prevented in most cases. The initiation of lactation was usually delayed but not entirely inhibited. When lactation had been established the amount of engorgment was not noticeably altered by stilbestrol treatment, however, milk secretion was appreciably diminished. R.P.R.

853. Studies on Pituitary Lactogenic Hormone. III. Solubilities of Sheep and Beef Hormones. CHOH HAO LI, WILLIAM R. LYONS AND HERBERT M. EVANS, Univ. California. Jour. Gen. Physiol., *24:* 303. 1941.

The solubility of sheep pituitary lactogenic hormone in 0.302 N NaCl at pH 2.02 (solution in HCl) was determined at room temperature. The hormone showed a constant solubility in the presence of a considerable excess of solid phase, indicating that the preparation contained but one component. Beef lactogenic hormone showed a constant solubility in distilled H_2O at 7–8° C. in the presence of excess of the solid phase. The salting-out effect of NaCl in acid solution of both sheep and beef hormones was studied at room temperature. Both preparations behaved as pure substances, but they exhibited differences in solubility, thus indicating a species specificity. R.P.R.

854. Inhibition of Lactation. Percutaneous Use of Testosterone. ABRAHAM J. FLEISCHER AND J. IRVING KUSHNER, Bronx Hospital, New York City. Jour. Clin. Endocrinol., *1:* 407. 1941.

An attempt was made to inhibit lactation in 100 postpartum women with

testosterone propionate. Four mg. were administered by inunction daily, beginning on the second postpartum day. (The authors fail to mention the number of daily administrations employed.) The treatment was considered effective in 68 per cent of the cases. It was partially effective in 29 per cent of the cases and was entirely without effect in 3 per cent of the cases. In not one case was lactation entirely arrested, the effect being a diminution in the degree of lactation with resulting diminution of secondary signs and symptoms. R.P.R.

855. Does Pregnancy Suppress the Lactogenic Hormone of the Pituitary. C. W. Turner and Joseph Meites, Univ. Missouri. Endocrinology, *29:* 165. 1941.

Experiments were carried out in an attempt to determine whether or not there was an actual suppression of the lactogenic hormone during pregnancy. The lactogen content of the pituitaries of 10 lactating rabbits 20 days postpartum was similar to that of a group of 10 lactating and pregnant rabbits, 20 days postpartum. The average lactogen content per pituitary of rabbits ovariectomized on the 20th day of pregnancy and killed 2 to 5 days later was not significantly different from the amount present in the pituitaries of intact animals on the 20th day of gestation. The lactogenic hormone was detected in the placentas of 3 of the pregnant-lactating group. The authors conclude that pregnancy has no inhibitory effect on the lactogenic hormone of the pituitary. R.P.R.

856. Influence of Epinephrin and Adrenal Cortical Extract on the Lactogenic Properties of Prolactin. David R. Climenko and Evans W. McChesney, Res. Labs., Winthrop Chemical Co., Rensselaer, N. Y. Endocrinology, *28:* 710. 1941.

A study was made of the effect of epinephrin and an adrenal cortical extract (Wilson and Company) on the lactogenic response of the lactogenic hormone. A series of 158 albino rats were rendered pseudo-pregnant by the administration of chorionic gonadotrophin. A slight degree of secretory activity was induced in the mammary glands by the treatment and the administration of 50 I.U. of the lactogenic hormone considerably augmented the effect. A somewhat greater effect was produced by 100 I.U. of lactogen but no significant increment of glandular activity was noted by raising the dose to 150 I.U. The secretory activity obtained by the administration of 50 I.U. of lactogen was somewhat augmented by the simultaneous administration of adrenal cortical extract. A considerably greater effect was obtained if the lactogen treatment was accompanied by an epinephrin-induced state of hyperglycemia. Supplementation by both cortical extract and epinephrin did not seem to produce as great an effect as that produced by supplementation with epinephrin alone. It was suggested that the effect of

the cortical extract might possibly be explained on the basis of its anti-insulin properties. R.P.R.

857. "Mammogen" and the Treatment of Spayed Hypophysectomized Rats with Lipoid Extracts of Cattle Pituitary. Roy O. Greep and Homer E. Stavely, Div. of Pharmocology and Organic Chemistry, Squibb Inst. for Med. Res., New Brunswick, N. J. Endocrinology, *29:* 18. 1941.

Female cattle pituitaries desiccated in vacuo, powdered, and injected as a suspension in saline induced mammary growth in spayed hypophysectomized rats. A lipoid fraction, however, produced none of the signs of mammary stimulation. The mammogenic activity of the tissue residue was somewhat reduced by the extraction procedure but this loss of activity was not accounted for in the lipoid fraction. The pituitary tissue, before and after extraction, produced body growth. Increases in body weight and the extent of mammary stimulation were not always correlated. R.P.R.

858. Effect of Stilbestrol on the Mammary Gland. A. A. Lewis and C. W. Turner, Univ. Missouri, Columbia, Mo. Amer. Soc. Anim. Prod. Proc., *33:* 63–66. 1940.

Stilbestrol, a synthetic chemical, was reported to have the ability to stimulate the growth of mammary ducts, and some lobule-alveolar tissue, but differs from estrogen in having ability in addition to stimulate milk secretion in virgin or dry animals. It did not consistently stimulate animals already in lactation. G.C.W.

859. The Time of Ovulation and Rate of Spermatozoa Travel in Cattle. J. E. Brewster, R. May and C. L. Cole, Michigan State College, East Lansing. Amer. Soc. Anim. Prod. Proc., *33:* 304–311. 1940.

The average time of ovulation was 13.57 ± 0.68 hours from the end of estrus in 53 cases of beef and dairy cows as determined by palpation. Heifers ovulated 3.04 hours earlier than cows that had previously calved. Ovulation occurred at any time during the day or night. Some of the cows were slaughtered after artificially inseminating with a bull producing active spermatozoa. Measurements on different parts of the genital organs revealed that the length of the tract was definitely longer for cows than for heifers. The average time required for sperm to reach the upper tube in cows was 5½ hours and in heifers 4¼ hours. The rate of travel appeared to be slightly slower in beef than in dairy cows. G.C.W.

860. The Surgical Anatomy of the Teat of the Cow. H. L. Faust, Dept. Vet. Anat., Iowa State College. Jour. Amer. Vet. Med. Assoc., *98:* 143. 1941.

The anatomical structure of the bovine teat is carefully described in a

paper which is well illustrated with pictures and drawings. Schematic drawings of several of the stages in embryonic development of the teat are also shown. F.E.

MISCELLANEOUS

861. The Competitive Position of Dairying in Michigan. Ross V. Baumann and E. B. Hill, Mich. Agr. Expt. Sta., East Lansing, in cooperation with the U. S. D. A. Bur. of Agr. Econ., Spec Bul., 209. June, 1941.

This study was made in the effort to provide information concerning the outlook for dairying in Michigan. The advantages of dairying, compared with other lines of production, under conditions likely to develop in the future are considered. Estimates of future production are made over a ten-year period under different price conditions and also assuming there is no change in price relationships. Two areas were studied in detail and the results extended to surrounding regions by comparisons of farming systems and other conditions.

A continuation of the present trend toward more milk production is indicated by a budget analysis of individual farms. Additional feed is expected to become available as a result of greater use of alfalfa and hybrid corn. P.H.T.

862. How to Figure Refrigeration Insulation. Refrigeration Engineering Application Data 27. Lee C. Leslie, Philadelphia, Pa. Refrig. Engin., *42*, No. 2: 136. 1941.

The author gives details in arriving at economic thicknesses of insulation citing practical problems and accompanying his explanations with tables and graphs. Attention is given to moisture resistance and protection and proper methods of installation of slab and bulk insulation materials. Numerous tables of valuable material are also included. L.M.D.

863. Locker Storage and Related Freezing Facilities for Community Storage Plants. W. R. Woolrich, Univ. Texas, Austin, Texas. Ice and Refrig., *100*, No. 3: 199. 1941.

This paper traces the historical development of locker storage plants and also announces the development by the University of Texas Bureau of Engineering Research of a new freezer for quick freezing of vegetables and fruits at a cost of 2½ cents (including preparation of the product) per pound. A description of the types of locker plants is included. Some plants offer a multiplicity of services, *e.g.*, (1) Slaughtering of livestock, (2) chilling of meats, (3) curing of meats, (4) slicing and wrapping of meats, (5) sausage

making and lard rendering, (6) smoking of meats, (7) fruit, vegetables, and fish processing, (8) sharp freezing, (9) quick freezing, (10) storage and (11) selling and buying of products.

A discussion of plant design is included. The chill room should be at least 12 ft. high and be insulated for a temperature of 30° F. This calls for 5 or 6 inches of cork or its equivalent. The area of the chill room should be about $\frac{1}{2}$ sq. ft. per anticipated locker capacity. The outside wall and ceiling of the sharp freezer room should have 8 inches of cork or its equivalent while the inner walls between locker, chill room and processing rooms should have at least 6 inches since this room is designed for a minus 15° F. temperature. It should have a minimum of 60 sq. ft. of floor space for a 300 locker plant plus $\frac{1}{10}$ sq. ft. for each additional locker. The locker room temperature should be near 0° F. in order to minimize odors. The aisles should be 30 inches wide. Ordinarily the dimensions should be 2.5 to 3 sq. ft. per locker installed. Load concentrations on the floor may reach 400 to 500 lbs. per sq. ft. Odorless asphalt should be used in the construction of locker rooms.

Refrigeration requirements average 1 ton per 50 lockers in the south or per 60 lockers in the north. L.C.T.

864. The Conservation of Water in Refrigeration. GEORGE E. TOLES. Ice and Refrig., *100*, No. 2: 109. 1941.

This article resolves itself into a justification for the use of evaporative condensers in preference to all other kinds as a means for cutting down on condensing water costs. Figures are given for relative savings on a 50 ton plant if different condensers are used. It is pointed out that with a cooling tower 4 gallons of water per minute per ton capacity must be circulated, while an evaporative condenser will use but $1\frac{3}{4}$ gallons of water per hour plus a 25 per cent entrainment. In the case of a city water condenser, 1 gallon per minute per ton is the usual usage. Even though air circulating fans are required with the latter system, the final operating cost will probably be 25 per cent that of the city water system and 35 per cent of that of the cooling tower system. Proper engineering can make the evaporative condenser fully automatic in operation.

Where the evaporative condenser is not used, savings in water may be effected by using the condenser water for other purposes. L.C.T.

865. Census Bureau Reports on Refrigerating Business. ANONYMOUS. Ice and Refrig., *100*, No. 2: 97. 1941.

The article in question consists entirely of a mass of data giving production figures and values of mechanical units and value of ice manufactured. Machines are classified according to power usage. Employment figures for the refrigerated machinery industry are given. L.C.T.

866. Construction of Flooded Evaporators for Stable Operation. F. J. Bobby, The Union Ice Co., Watsonville, Calif. Ice and Refrig., *100*, No. 1: 32. 1941.

Flooded evaporators should meet certain requirements, *e.g.:*

1. The unit must be arranged in such a way as to operate with a low liquid head to obtain the lowest temperatures of the refrigerant for any given evaporating pressure.

2. It must produce proper circulation of the liquid refrigerant in order to wash the gas film from the transfer surfaces.

3. It must permit proper refrigerant velocities in the pipe coils in order to avoid excessive friction and turbulence.

4. It must be arranged in such a way as to provide the proper liquid head in order to give this desired circulation.

5. It must be provided with adequate return pipes for the circulating liquid (drop legs) in order to return the circulating liquid to the evaporator and avoid piling up under maximum load conditions.

6. It must provide either fixed or variable liquid level control in order to fix the operating head under any given load and to protect against flooding.

The author points out that 50 per cent vapor to total gas and liquid by volume is about optimum. A velocity of 300 ft. per minute in the piping is desirable. In order to maintain the proper velocity a certain amount of head must be maintained. This can be determined by use of certain formulae which the author includes. Operating diagrams also are given. L.C.T.

867. Piping Design Problems in Frozen Foods Locker Plants. John H. Carter, Kupferle-Hicks Heating Co., 3974 Delmar Blvd., St. Louis, Mo. Ice and Refrig., *99*, No. 4: 271. 1940.

This article includes a floor plan for a 415 locker plant. Temperatures and times which are most desirable are listed as follows: Chilling, 33° to 35° F. for about 24 hours; aging, 33° to 35° F. for 3 days for hogs, ordinary beef a week to 10 days, and well fattened corn-fed steer beef as much as three or four weeks; freezing, – 15° F. as low as 12 minutes for peas, and as much as 12 hours for a large roast; storage, 0 to 12° F. with 0° to 5° most desirable, and average time about 3 months. The usual loading allowance is 3 pounds of product per locker per day, somewhat less in Summer, and somewhat more in Fall. For a 415 locker plant as illustrated, the estimated load for the locker room alone is 10,878 btu. per hour maximum. The locker room would require about 800 ft. of 1¼ inch F.W.B.S. piping, or about 350 sq. ft. of coil surface. If the coil surface is free from frost and a transfer factor of 2.5 btu./hr./sq. ft./deg. temp. difference is assumed, a temperature difference of 12.5° F. must be maintained. As frost accumulates this difference must be increased or the coil area must be increased beyond 800 sq. ft., *e.g.*, to 1200 sq. ft.

For the freezer room with a maximum requirement of 16,625 btu./hr., about 200 sq. ft. of piping will be required if forced circulation at the rate of 500 ft./min. is used, and the transfer factor is 7.0. About 8 to 12 air changes per minute are recommended. The capacity of the blower can then be readily estimated. Coils should be defrosted frequently. About 600 ft. of 1¼″ F.W.B.S. pipe coil are advised for the aging room. This is based on a 4221 btu./hr. load. The chill room requires about 435 ft. of 1¼″ F.W.B.S. pipe coil, based on a 3011 btu./hr. load. The general storage room with a 3515 btu./hr. load will take about 325 ft. of 1¼″ F.W.B.S. pipe coil. With a load of 2902 btu./hr. the curing room will require about 205 ft. of 1¼″ F.W.B.S. pipe coil.

All figures in this article were based on the use of ammonia as the refrigerant, and would require revision if others were used. L.C.T.

868. Priorities and Your Business. ANONYMOUS. Ice Cream Field, *38*, No. 2: 39. 1941.

Mention is made of the fact that many products, including aluminum, nickel-bearing steel, milk steel, rubber, synthetic rubber, and electric motors are now on the priority list of the Office of Production Management in Washington. Other additions are being made to this list and the Dairy Industries, themselves essential industries, are finding it more difficult to operate.

Those responsible for the nation's planning realize the importance of dairy products to the health and well being of her population and are doing what they can to enable the Dairy Industries to continue to operate although very definite restrictions and changes have been made in supplies and equipment, and others will of necessity follow. So far many of these restrictions are on a voluntary basis and it is stated that if handled properly this can continue.

One of the demands of the O.P.M. is that all industries attempt to work out suitable substitutes to relieve shortages of critical materials. For the industrial equipment and supplies field this program, it is stated, has been assumed by a special sub-committee of the Dairy Industries Supply Associations Technical Committee. W.C.C.

869. Raising the 4-H Dairy Calf. Raising the 4-H Dairy Heifer. Managing the 4-H Dairy Cow. E. J. PERRY AND J. R. PORTER. New Jersey Agr. Ext. Serv. Bul. 225, 226, and 227, respectively. 11 pages, 14 pages, and 18 pages, respectively. 1941.

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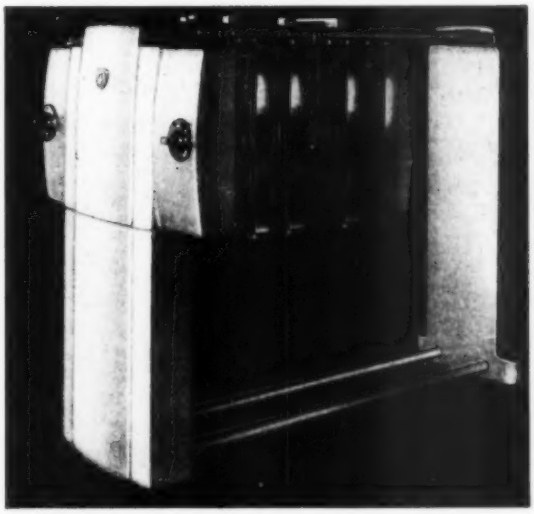

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DIFCO

DETECTION OF *Coli* IN MILK

BACTO-VIOLET RED BILE AGAR

is recommended in "Standard Methods for the Examination of Dairy Products" of the American Public Health Association for direct plate counts of coliform bacteria in milk and other dairy products. Upon plates of medium prepared from Bacto-Violet Red Bile Agar coliform organisms form reddish colonies, 1 to 2 mm. in diameter, which are usually surrounded by a reddish zone of precipitated bile. After incubation for 18 hours at 37°C. counts of these colonies may be made without interference by extraneous forms.

BACTO-BRILLIANT GREEN BILE 2%

is an excellent medium for detection of the presence of coliform bacteria in milk. Results obtained by direct inoculation of milk into fermentation tubes of medium prepared from Bacto-Brilliant Green Bile 2% are a dependable indication of the presence of coliform organisms in the original sample. Use of this dehydrated medium is approved in "Standard Methods."

BACTO-FORMATE RICINOLEATE BROTH

is also a useful medium for detection of coliform organisms in milk. The medium is used in fermentation tubes which are inoculated with the milk sample. Growth of lactose fermenting bacteria is stimulated and gas production is accelerated in this medium. Use of the dehydrated medium is approved in "Standard Methods."

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